Original Article

Protective effects of forced exercise against methylphenidate-induced anxiety, depression and cognition impairment in rat

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Abstract

Background: Methylphenidate (MPH), a neural stimulant, can cause damages to brain; the chronic neurochemical and behavioral effects of MPH remain unclear. Exercise lowers stress and anxiety and can act as non-pharmacologic neuroprotective agent. In this study protective effects of exercise in MPH-induced anxiety, depression and cognition impairment were investigated.

Materials and Methods: Seventy adult male rats were divided randomly into five groups. Group 1 served as negative control, received normal saline (0.2 ml/rat) for 21 days, group 2 and 3 (as positive controls) received MPH (10 and 20 mg/kg) for 21 days. Groups 4 and 5 concurrently were treated with MPH (10 and 20 mg/kg) and forced exercise for 21 days. On day 21, Elevated Plus Maze (EPM), Open Field Test (OFT), Forced Swim Test (FST) and Tail Suspension Test (TST) were used to investigate the level of anxiety and depression in animals. In addition between 17th and 21th days, Morris Water Maze (MWM) was applied to evaluate the effect of MPH on spatial learning and memory.

Results: MPH-treated animals indicated a reflective depression and anxiety in a dose-dependent manner in FST, EPM and TST which were significantly different from the control group and also can significantly attenuate the motor activity and anxiety in OFT. Forced exercise by treadmill can attenuate MPH-induced anxiety, depression and motor activity alteration in OFT. MPH also can disturb learning and memory in MWM and forced exercise can neutralize this effect of MPH.

Conclusion: We conclude that forced exercise can be protective in brain against MPH-induced anxiety, depression and cognition alteration.

Key Words: Anxiety, cognition impairment, depression, forced exercise, methylphenidate

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INTRODUCTION

Methylphenidate (MPH: Ritalin) is a neural stimulant prescribed for the management of attention-deficit/hyperactivity disorder.[1,2] The long-term neurochemical and behavioral effects of MPH treatment remain unclear.[3,4] MPH binds the dopamine and to a lesser extent norepinephrine transporters, blocking the

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reuptake of both neurotransmitters into synaptic terminals, and therefore enhancing their effects on receptors.[2,5] MPH has high potential for abuse and addiction due to its structural and functional similarity to cocaine and amphetamines.[6] A little information exist on chronic effects of MPH.[6,7] Previous studies confirmed that chronic administration of MPH can induce neurodegeneration in some areas of brain such as hippocampus and amygdale.[8,9] Behavioral alterations which are seen after chronic use of MPH consist of increases in depressive- and anxiety-like behaviors and also decrease in spatial learning and memory.[10-12]

Previous studies have also demonstrated that exercise lowers stress and anxiety and increases endorphin secretion in brain.[13,14] Physical activity improves anxiety symptoms in healthy people and patients; chronic forced exercises produce anxiolytic-like effects in some experiments.[15] It has also been shown that exercise can counteract with depression and can increase the cognitive function.[16,17] Chronic exercise in mice results in antidepressant-like behavioral changes that may involve a BDNF-related mechanism similar to that hypothesized for antidepressant drug treatment.[18] Exercise increases synthesis and release of dopamine, stimulating neuroplasticity and promotes feelings of well-being.[18,19] In the present study the protective effects of exercise in MPH-induced anxiety, depression and cognition impairment were investigated.

MATERIALS AND METHODS

Animals

Seventy adult male rats, average weight 220 g, and 9 weeks old obtained from animal house of Iran University of Medical Sciences (Tehran, Iran) and transferred to the lab. They were kept for 2 weeks at room temperature with free access to standard food and tap water and with standard cycle of Light and dark. Our experimental protocol was approved by the Research Council of the Iran University of Medical Sciences.

Drug

MPHs were purchased from Shafayab Gostar Co (Tehran, Iran) and Daru Pharmaceutical Company and were freshly prepared just before use.

Experimental design

Group 1 was treated with normal saline (0.2 ml/rat) for 21 days and served as negative control. Groups 2 and 3 (served as positive controls) received MPH (10 and 20 mg/kg) for 21 days. Groups 4 and 5 were treated by MPH (10 and 20 mg/kg) and forced exercise concurrently for 21 days.

On day 22, some standard behavioral methods such as Elevated Plus Maze (EPM), Open Field Test (OFT), Forced Swim Test (FST) and Tail Suspension Test (TST) were used to investigate anxiety and depression level in animals. In addition, Morris Water Maze was used to evaluate spatial learning and memory in animals between 17th and 21th day.

Treadmill forced exercise protocol

Rats were allowed to run on a motor-driven leveled treadmill (Model T408E, Diagnostic and Research Instruments Co., Taoyuan, Tai). The animal of Groups 4 and 5 were trained by treadmill for 45 min/day, for 5 days/week. The training speed was 12 miles per minute (for first week) and reached 14 miles per minute (in the second week) by the end of the experiments. The slope and intensity of exercise was settled as 0º at the first 10 min, 5º for second 10 min and 15º for last 25 minutes.

Behavioral tests

Open field test

This equipment was used for assessment of anxiety and locomotor activity of rodents. The bottom of this apparatus was divided into 16 equally spaced squares bordered by opaque high walls of 65.90 cm. The entire equipment was dyed black except for the 6 mm broad white lines that divided the ground into 16 squares. The open field was illuminated by a 100 W bulb focusing into the field from a height of about 110 cm from the ground. The whole part of room except the open field was reserved dark during the experiment. For assessment of anxiety and locomotor activity, each animal was centrally located in the test apparatus for a maximum of 5 minutes to monitor the subsequent behaviors.

- Ambulation distance: Distance which rat crossed of the grid lines
- Center square entries: Quantity which the rat crossed one of the central red lines with all four paws and inter into the central square
- Center square duration: The time the rat spent in the central square
- Rearing: Quantity with which the rat stood on their hind legs in the maze.

Forced swim test

This apparatus commonly applied for evaluation of depressant like behavior in rodents. The apparatus was composed of a transparent Plexiglas cylinder with 25 cm diameter and 60 cm height which filled with water to height of 20 cm. For adaptation of animal with test condition all rats individually were subjected to forced swim for a period of 15 min, 1 day before the actual test; on the day of experiment, the animals were

located individually in water-filled cylinder for a period of 6 minutes. The duration of swimming was recorded in period of 6 minutes. Swimming activity is produced during prolonged periods of non-depressive behavior.

Elevated plus maze

Another test which was applied for anxiety in rodents is Elevated Plus Maze (EPM). The equipment composed of two opposite arms 55 × 15 cm, which connected with a central square (10 × 10 cm), this apparatus shape was plus sign. One arm was reserved open, while other arm was enclosed with the 40 cm elevated wall. Entire apparatus was reserved elevated 50 cm above the ground. All animals were located individually in the center of the maze in front of an enclosed arm and the time which the animal spent on the open arms were recorded during 5 min for each rat. More time spent in open arm indicates non-depressive behavior

Tail suspension test

In this test, animal hang up from tail with tape which stick to 4/5 of the tail length and suspended from a metal rod which was fixed 50 cm above the surface area. The duration of immobility and heave of animal was recorded for 5 minutes period. Immobility is considered as depressive like behavior.

Morris water maze task

MWM was composed of a circular black-colored water tank (150 cm in diameter and 85 cm in height) which was set up in the center of small room. This apparatus was divided into four quadrants (North, East, West and South). The tank was filled by water to the height of 50 cm. The operator stay in the North-East part of the room. A platform disk with 12 cm diameter (made of Plexiglas) which is invisible was inserted 1 cm beneath the surface of the water. In the first 4 days of experiment, which called training procedure the platform was located constantly in one of the quarter. An automated infrared tracking system (CCTV B/W camera, SBC-300 (P), Samsung Electronic Co., Ltd, Korea) recorded the position of the animal in the tank. The camera was mounted 2.3 m above the surface of the water.

Handling

Before the start of experiment, on the first day, rats were located on the tank that was filled with water, room temperature (25 ± 2 ºC) and the operator guided the rat for swimming to reach to the platform placed quarter. The platform was located on South-West quarter of the tank.

Training procedure

Some distinguish landmarks (such as picture, window, door, etc.) as set up in extra maze in the room for spatial cues for learning of platform's position for animals. The position of the platform was settled in the South-West quarter of MWM tank with 25 cm distance from the edge of the tank, and 1 cm beneath the surface of water. Each rat was experimented for four trials in a day. Each animal was tested randomly from four quarters (North, East, West and South). If the rats found the platform within the 60 s, the trial was automatically stopped by computer. In this experiment two parameters were evaluated.

- Time to find the hidden platform which was called escape latency And
- Distance traveled to the hidden platform which was called traveled distance were recorded.

On the fifth day, probe day, platform was removed and animal was thrown into water from one of the above-mentioned directions (East) and the percentage of presence of animal in the target quarter (South-West quarter) was recorded.

Statistical analysis

The data were analyzed by Graph Pad PRISM V.6 Software and averaged in every experimental group and expressed as Means ± Standard error of the means (SEM). Then the differences between control and treatment groups were evaluated by ANOVA. To evaluate the severity of behaviors, the differences between the means in each group were compared using the Tukey test at a significant level ($P < 0.05$).

RESULTS

Behavioral teratology in open field test

Results of open field test in control and treatment groups

As shown in Table 1 the negative control group in comparison with MPH-treated group with doses 10 and 20 mg/kg has more frequency of central square entries and also spent more time in the central region of the OFT ($P < 0.05$). Our study indicates that forced exercise in combination with MPH with doses of 10 and 20 mg/kg had more frequency of central square

Table 1: Effect of forced exercise training on open field exploratory and anxiety like behavior in rat under treatment by 10 and 20 mg/kg of methylphenidate

Group	Ambulation distance	Central square entries	Time spent in central square	Number of rearing
Control	435±15	22±2	160±10	13±2
MPH (10 mg/kg)	400±16[a]	15±1.5[a]	115±8[a]	7±1[a]
MPH (20 mg/kg)	370±19[a]	12±1[a]	100±11[a]	5±1[a]
Exercise+MPH (10 mg/kg)	420±25[b]	18±1.2[b]	145±10[b]	9±1[b]
Exercise+MPH (20 mg/kg)	400±25[c]	16±1[c]	137±12[c]	8±2[c]

[a]$P<0.05$ vs. control groups, [b]$P<0.05$ vs. 10 mg/kg of methylphenidate and [c]$P<0.05$ vs. 20 mg/kg of methylphenidate. MPH: Methylphenidate

entries and also spent more time in the central region of the OFT. This difference was statistically significant in comparison with MPH (10 and 20 mg/kg)-treated group ($P < 0.05$) [Table 1].

Negative control animals in comparison with the MPH-treated group with doses 10 and 20 mg/kg had more frequency of ambulation number and rearing and ambulation distance in OFT ($P < 0.001$). Also animals treated by MPH (10 and 20 mg/kg) and forced exercise concurrently had more frequency of ambulation, rearing and ambulation distance in OFT. This difference was statistically significant in comparison with MPH (10 and 20 mg/kg)-treated group ($P < 0.05$) [Table 1].

Results of forced swim test in control and treatment groups

The MPH-treated group with doses 10 and 20 mg/kg in comparison with the negative control group, spent less time swimming in the FST with $P < 0.05$ [Figure 1].

This research indicated that animals treated with MPH with used doses in combination with forced exercise, spent more time swimming in the FST in comparison with the MPH (10 and 20 mg/kg) alone treated group ($P < 0.05$) [Figure 1].

Results of elevated plus maze in control and treatment groups

The MPH-treated group with doses 10 and 20 mg/kg spent less time in open arms in comparison with the negative control group in EPM ($P < 0.05$) [Figure 2].

This research indicated that animals treated with MPH with doses 10 and 20 mg/kg in combination with forced exercise, spent more time in open arms in EPM in comparison with the group treated with MPH (10 and 20 mg/kg) alone ($P < 0.05$) [Figure 2].

Results of tail suspension tes in control and treatment groups

Mean duration of immobility in the MPH-treated group with doses 10 and 20 mg/kg was significantly increased compared to animals in the control group in TST ($P < 0.05$). A decrease in immobility due to forced exercise in the MPH-treated group with doses 10 and 20 mg/kg was found to be statistically significant ($P < 0.05$) [Figure 3].

Evaluation of escape latency and traveled distance during training days in the morris water maze

Mean of escape latency and traveled distance during 4 days training in the MWM for group treated by MPH (20 mg/kg) was statistically significant in comparison with the negative control group ($P < 0.05$) [Figures 4 and 5].

The MPH (20 mg/kg)-treated group concurrently by forced exercise showed a significant decrease in mean of escape latency and traveled distance during 4 days training in the MWM in comparison with group treated by MPH (20 mg/kg) alone ($P < 0.05$) [Figures 4 and 5].

Evaluation of swimming speed during training days

The swimming speed was not altered during training trials in any of the animal groups, suggesting that exposure to MPH alone with doses 10 and 20 mg/kg or in combination with forced exercise did not cause any motor disturbances in animals under study [Figure 6].

Evaluation of percentage in target quarter in probe trial

Results showed that there was a significant difference in percentage of the presence of animals in target quarter in the MPH (20 mg/kg)-treated group in comparison to the negative control group ($P < 0.05$) [Figure 7]. Also, the difference between group treated by forced exercise and MPH (20 mg/kg) stayed longer in target quarter

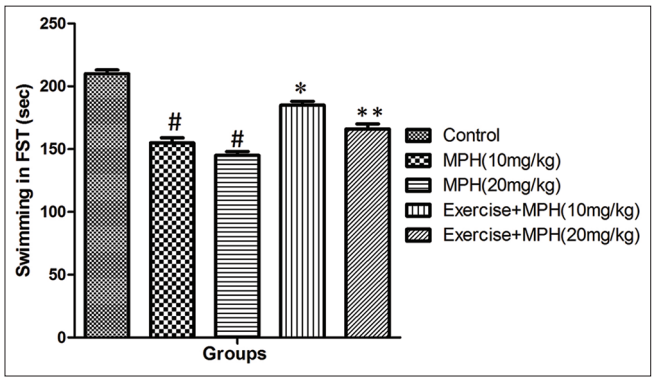

Figure 1: Swimming time (seconds) in Forced Swim Test (FST) in the control group, and groups under treatment with 10 and 20 mg/kg of Methylphenidate and the same doses of methylphenidate in combination with forced exercise. All data are expressed as Mean ± SD (n=8). * P< 0.05 vs10 mg/kg of methylphenidate. **P< 0.05 vs. 20 mg/kg of methylphenidate. #P< 0.05 vs. control groups. MPH: Methylphenidate

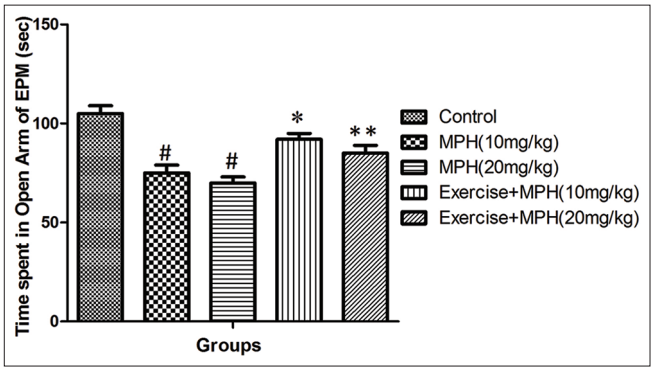

Figure 2: Duration of time spent in open arms (seconds) in Elevated Plus Maze(EPM) test in the control group and groups under treatment with 10 and 20 mg/kg of methylphenidate and the same doses of methylphenidate in combination with forced exercise. All data are expressed as Mean ± SD (n=8). * P< 0.05 vs. 10 mg/kg of methylphenidate. **P< 0.05 vs. 20 mg/kg of methylphenidate. #P< 0.05 vs. control groups. MPH: Methylphenidate

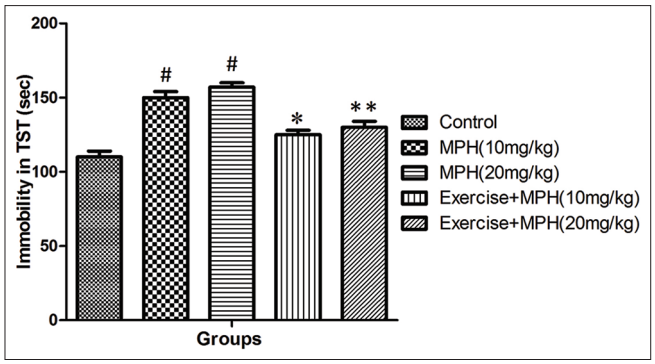

Figure 3: Duration of time stayed in immobility (seconds) in Tail Suspension Test(TST) in control group and groups under treatment with 10 and 20mg/kg of Methylphenidate and the same doses of Methylphenidate in combination with Forced exercise. All data are expressed as Mean ± SD (n=8). *P< 0.05 vs. 10 mg/kg of methylphenidate. **P< 0.05 vs. 20 mg/kg of Methylphenidate. #P< 0.05 vs. control groups. MPH: Methylphenidate

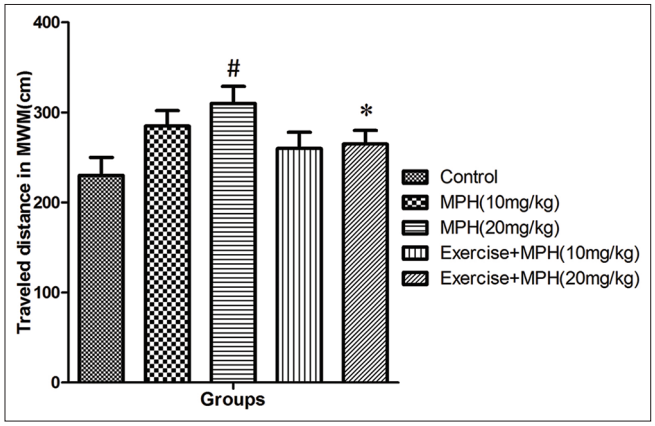

Figure 5: Average of traveled distance in control group and groups under treatment with 10 and 20mg/kg of Methylphenidate and the same doses of Methylphenidate in combination with Forced exercise across all training days using Morris Water Maze (MWM) in rats. Data are shown as means ± SD. *P< 0.05 vs 20 mg/kg of Methylphenidate. #P< 0.05 vs control groups. MPH: Methylphenidate

in comparison with the methylphenidate (20 mg/kg) alone treated group (P < 0.05) [Figure 7].

DISCUSSION

The present study indicates that forced exercise can be modulating MPH-induced anxiety, depression and cognition alteration. MPH administration resulted in significant behavioral alterations in FST (swimming and immobility) and EPM (open arm and close arm entry). MPH in various doses can also alter behavioral parameters in OFT (central area entry, central area duration, ambulation and rearing). High doses of MPH can suppress cognitive behavior such as spatial learning and memory. MPH is a neural stimulant agent that is in use for management of hyperactivity in attention –deficit/ hyperactivity disorder in children.[2] This agent

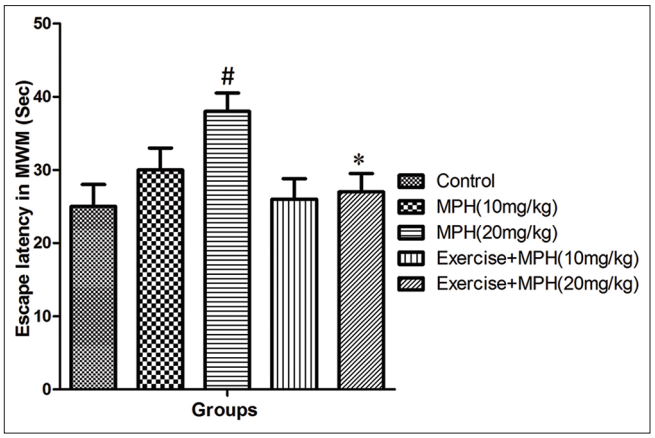

Figure 4: Average of escape latency in control group and groups under treatment with 10 and 20 mg/kg of methylphenidate and the same doses of methylphenidate in combination with forced exercise across all training days using Morris Water Maze (MWM) in rats. Data are shown as means ± SD. *P< 0.05 vs. 20 mg/kg of methylphenidate. #P< 0.05 vs. control groups. MPH: Methylphenidate

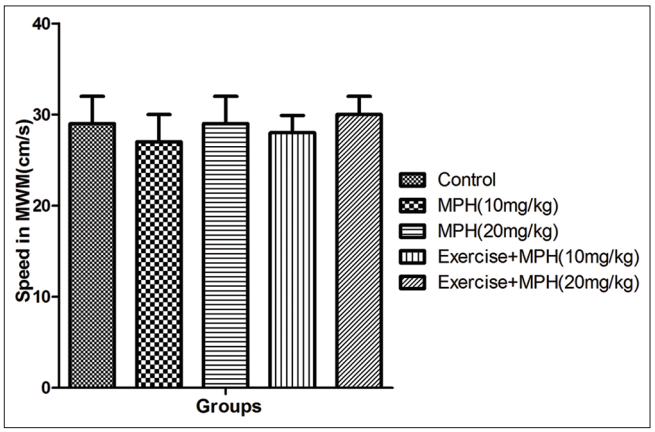

Figure 6: Average of swimming speed in control group and groups under treatment with 10 and 20 mg/kg of methylphenidate and the same doses of Methylphenidate in combination with Forced exercise across all training days using Morris Water Maze (MWM) in rats. Data are shown as means ± SD, MPH: Methylphenidate

acts like methamphetamine compounds such as methylene dioxy methamphetamine (MDMA).[2,20] MPH binds the dopamine transporter, and to a lesser extent the norepinephrine transporter, and inhibits reuptake of both neurotransmitter to presynaptic terminals,[21,22] but the long-term and neurobehavioral consequences of MPH treatment in pharmacologic and non-pharmacologic doses are unknown.[4] Previous studies have revealed that physical activity lowers stress and anxiety levels and releases endorphins into the brain.[23,24] Exercise can counteract drug abuse withdrawal symptoms by attenuation of depression, reducing anxiety and helping the patient to feel better.[23] Several research studies have demonstrated that exercise can manage this recovery process. Physical activity can reduce the risk of relapse in drug abuse.[25] Previous study demonstrated that

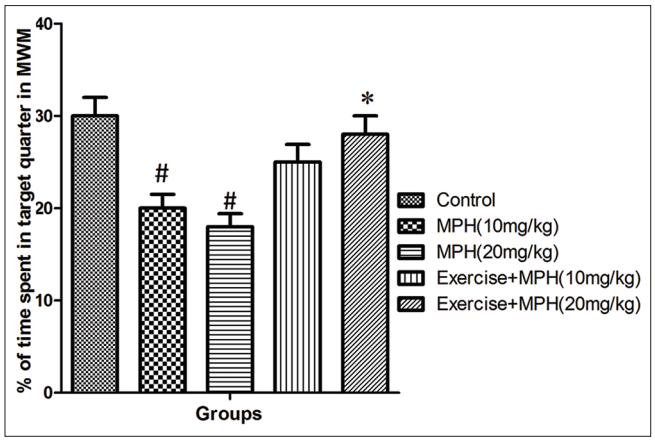

Figure 7: Percentages of time spent in target quarter in probe trial in the control group and groups under treatment with 10 and 20 mg/kg of methylphenidate and the same doses of methylphenidate in combination with forced exercise across all training days using Morris Water Maze (MWM) in rats. Data are shown as means ± SD. **P*< 0.05 vs. 20 mg/kg of methylphenidate. #*P*< 0.05 vs. control groups. MPH: Methylphenidate

exercise reduces the rewarding effects of some drugs such as cocaine and morphine since exercise leads to an increase in the synthesis and release of dopamine, stimulates neuroplasticity and promotes feelings of well-being.[26,27] The present study demonstrated that MPH with doses of 10 and 20 mg/kg cause decrease time of swimming in FST. The period was statistically significant in comparison with the control group. This study indicated that MPH cause depression like behavior in an animal model. In addition, our data showed that forced exercise can suppress the effect of MPH with doses of 10 and 20 mg/kg and increase the time of swimming in FST. Our data is consistent with previous study that forced exercise can modulate depression and anxiety. We can discuss our finding with basic concept that MPH as methamphetamine like compound can alter brain monoamines and can induce anxiety and depression.[28] Previous findings suggest that MPH treatment during adolescence induces persistent changes on emotionality and behavior.[12] On the other hand, the previous study indicated that exercise and physical activity can improve depression and anxiety. These data suggested that exercise can increase the brain monoamines and increase the level of serotonin and dopamine in brain thus it can be modulating depression and anxiety.[29]

Our data demonstrated that MPH by doses of 10 and 20 mg/kg cause decrease in period of time spent in open arm of EPM. This decrease was statistically significant in comparison with the control group. Also, we showed that chronic uses for MPH-induce anxiety and fear-like behavior in an animal model. Based on our findings, forced exercise can abolish these effects of MPH and increase the time of animal presence in open arm in EPM. We can compare this data with previous study results showing the influence of MPH treatment in adult rats, which showed the increased anxiety, associated with the neural sensitization of anxiety-related behavior in EPM test after a sudden MPH treatment break.[30] Many studies demonstrated that forced and voluntary exercise can activate brain reward system; this study showed that physical activity abolish anxiety and depressive like behavior in rodents.[31]

In OFT, our study results showed that MPH with doses of 10 and 20 mg/kg cause decreases in ambulation distance which indicated MPH abuse can alter motor activity. Our data showed that physical activity can abolish this effect of MPH on motor activity and increase the ambulation distance. Some previous study demonstrated that MPH can increase motor activity in children but other indicated that MPH can decrease and disturb the motor activity in animal model.[30,32] On the other hand, a previous study showed that MPH can alter and normalize motor activity in opioid and other substance-abused animals. Based on mentioned study and our findings, we can argue that MPH can disturb the motor activity by altering the reward system and motor activity controlling center. Physical activity can diminish the side effects and normalize these effects.[18,32,33] MPH in mentioned doses can decrease central square entries and time spent in Central Square in OFT, and this decrease was statistically significant in comparison with the control group, but forced exercise in the MPH-treated group can alter and increase the central square entries and time spent in Central Square in OFT and this increase was statistically significant in comparison with the MPH (with doses of 10 and 20 mg/kg)-treated group. MPH causes decrease rearing number at both doses and this decrease was statistically significant in comparison with the control group, our data demonstrated that forced exercise attenuate this effect of MPH and increased the number of rearing which this effect of physical activity was statistically significant in comparison with the MPH (with doses of 10 and 20 mg/kg)-treated group.

According to FST and EPM, MPH can induce depression and anxiety and results of OFT verified this effect. We can argue this effect with basic concept that MPH like other methamphetamine-like compounds can alter brain dopamine and adrenaline levels and deplete them and thus induce depression and anxiety.[34] Physical activity can also alter this effect of MPH and attenuate and diminish depressive and anxiety like behavior by activating the reward system.[18]

TST which is one of the standard tests for evaluation of depression showed that MPH cause increasing in immobility time and physical activity alters this effect of MPH at mentioned doses and decreases the time of immobility. We can compare this result with previous study that MPH like other amphetamine compounds can induce hopelessness and cause behavioral apathy and depression.[4] But forced exercise can increase the incentives to stimulate animal to do natural activities and abolish depressive side effect of MPH.[4,18]

Results of our study demonstrated that MPH at doses of 20 mg/kg cause increase in escaped latency and traveled distance in MWM test in comparison with the control group. In fact, our study results confirmed pervious results that MPH at high doses can cause disturbance in learning process. Our data also showed that the use of forced exercise, physical activity can counteract this effect of MPH and decrease traveled distance and escaped latency in MWM test.

Our study also showed that animals treated only by MPH or MPH in combination with exercise in comparison with negative control has no effect on swimming speed in MWM test.[35]

In MWM test in probe days our study indicated that MPH in doses of 10 and 20 mg/kg cause a decrease in percentage of time spent in target quarter (quarter which the platform was inserted) and this attenuation was statistically significant in comparison with the control group. On the other hand forced exercise can increase the percentage of time spent in the target quarter in the MPH-treated group. But this effect was just significantly different in exercise and MPH treated by dose of 20 mg/kg in comparison with MPH alone treated by 20 mg/kg. Our results are consistent with previous study results which indicated that MPH as a methamphetamine-like agent can alter brain cognition center because of the property of the agent which can deplete brain dopamine and adrenalin and these two amines are important in cognitive activity and have a major role in learning and memory. On the other hand, one of the major benefits of physical activity is improvement of cognition. Actually forced exercise increases somewhat brain adrenalin which is important in long term potentiating and stabilizing the learning and memory.[33]

CONCLUSION

The results of the present study support the hypothesis that forced exercise may be beneficial against MPH induced depression, anxiety and cognitive impairment and is freely accessible non pharmacologic therapy for patients which abuse MPH and suffering from its cessation-induced anxiety, depression and cognition impairment. These data could be helpful in human MPH abusers. However, further studies are required with human subjects.

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