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Original Research

# Interaction between anxiolytic effects of magnesium oxide nanoparticles and exercise in adult male rat

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## Abstract

***Objective(s):*** In recent years, nanotechnology has produced new forms of materials that are more effective than their predecessors. Magnesium is an essential element in the human body and certain studies have proved that its deficiency can induce anxiety in animals. In this study, the effect of magnesium oxide nanoparticles (MgO NPs) on anxiety, related behaviors, and interaction between their effects and anxiolytic effect of the exercises were examined in comparison to the conventional MgO (cMgO).

***Materials and Methods*:** Adult male Wistar rats weighing 190±20 gr were divided into control groups (receiving saline, without physical activity), and exercise groups (receiving cMgO and/or MgO NPs (1 mg/kg i.p.) daily for 6 weeks with or/and without exercise). Exercise groups were performing their daily physical activity protocol 30 minutes after injection. At the end of period, an elevated plus maze apparatus was used to evaluate the anxiety (%pen arm time (%OAT) and %open arm entries (%OAE) and locomotor activity.

***Results***: Exercise significantly increased %OAT and %OAE ($P<0.05$). MgO NPs caused an increase in %OAT, while cMgO did not have any effect on %OAT or %OAE. There was no notable difference among anxiety parameters in exercise groups with or without taking MgO NPs.

***Conclusion***: It seems that the anxiolytic effect of exercise and MgO NPs has been mediated through common mechanisms that were a part of the anxiety process of the central nervous system.

**Keywords**: Anxiety, MgO, Nanoparticles, Physical activity

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## Introduction

Magnesium (Mg) is the second most prevalent element in the human body which is essential for neurophysiological processes (1). Some studies have shown that magnesium's deficiency could induce anxiety (2-4). Magnesium acts on many channels and receptors in the synaptic space. *N*-Methyl-*D*-aspartate (NMDA) blocks glutamate inotropic receptor, thereby reducing the effect of glutamate as an excitatory amino acid in anxiety-like behaviors (5). Gamma amino butyric acid (GABA) is one of the inhibitory neurotransmitters, whose level magnesium can change in the body (6, 7). Each of the above mentioned mechanisms can aid magnesium in inducing anxiety behaviors.

Previous studies have shown that exercise activities also affect emotional health. In humans, exercise has been associated with the improvement of the outcome of treatments for depression and anxiety (8, 9). Exercise activities cause a reduction in the anxiety of mice and rats, as measured in the open field, by elevated plus maze and the light-dark box (10, 11). Moreover, short-term exercise training, including resistance exercise trainings, can increase the signs and symptoms associated with anxiety (12).

It has also been shown that exercise activities decrease serum magnesium levels and induce hypomagnesaemia (13). In fact, some studies suggested that magnesium supplements in athletes could be compensative agents in hypomagnesaemia (13, 14).

With the development of nanotechnology, new forms of materials with high therapeutic activity were produced and applied as a new branch of nanotechnology named nanomedicine (15). Nano drugs can cross every barrier, especially in central nervous system. In this area, the blood brain barrier had limited drug penetration. It's a good agent for imaging, for making diagnoses, and in the drug delivery system (16-18). Some advantages of the new materials are prolonged half-time in the body circulation, and reducing repeated doses. Also, in lower amounts, potentiated effects with minimum side effects can be seen (19, 20).

Magnesium-based nanomaterial such as Nano MgO (as metal oxide nanoparticle) is used in medicine to treat pathological disorders such as bacterial infection, and also for drug delivery (21). MgO nanoparticles (NPs) are nontoxic, bio-degradable, and have few side-effects (21). But the efficacy of Nano MgO, as the new form of magnesium supplement in CNS disorder, especially on anxiety related behaviors, is not yet clear.

This is the first study on the effect of chronic administration of MgO NP and its conventional form (cMgO) on anxiety behaviors in the presence and absence of anxiolytic mechanisms induced by exercise.

## Material and Methods

### *Animals*

Male albino Wistar rats weighting 190±20 gr were purchased from the animal house of Joundi Shapor University of Medical Sciences in Ahvaz, Iran. Animals were kept in a room with 12/12 hour dark/light cycle and a temperature of 23±1 ºC. MgO NP (lolitech Co, Germany, particle size <50 nm) and cMgO (Merk Co, Germany, particle size >100 nm) was dispersed in 0.9% saline by ultrasonic bath for 20 minutes and shaken for 1 minute before each injection. All drugs were injected intraperitoneally (i.p.), 1 mg/kg in volume of 1 ml/kg, Control groups were only given 0.9% saline.

Rats were randomly divided into six groups: treadmill controls, which were only given 0.9% saline without doing exercise (Cont), treadmill runners, which were given 0.9% saline (Exe), a group that was only given MgO NP 1 mg/kg (MgO NP), a group that was only given cMgO 1 mg/kg (cMgO), a group that was given MgO NP and was allowed exercise activities, and a group that was given cMgO and was also allowed exercise

activities (MgO NP+ Exe or cMgO+ Exe). Training and injections took place during the light cycle. In runner groups, drugs were injected 30 minutes before doing any exercise activity. All procedures were carried out in accordance with the institutional guidelines for animal care, and used by Shahid Chamran University of Ahvaz

***Exercise paradigm***

The training protocol was a model of forced aerobic exercise (22). Treadmill runners ran on a motorized treadmill that has 8 lines. They ran 5 days per week for 6 weeks. The grade of the apparatus was fixed the whole time. During the first week, animals acclimate to the treadmill, by gradually increasing running time each day (learning stage). During the second and third weeks, time and speed were increased (overload stage), but during the fourth and sixth, both time and speed were kept consistent (consolidation stage).

***Elevated plus maze***

The wooden elevated plus maze consisted of two opposite open arms (50 cm -10 cm) and two closed arms (50 cm-10 cm with 40 cm walls) in the shape of a cross which was connected by a central square (10×10 cm). The maze was elevated 50 cm above the ground and subjects were placed in the center square facing an open arm, and were allowed to explore the maze for five minutes while their behavior was recorded by camera and analyzed by a maze router software. The observed behaviors included the percentage of open arm time (open/ (open + closed) %OAT), the percentage of open arm entries, (open/ open+ closed) %OAE), and the distance traveled in open and closed arms in 5 min, calculated as locomotor activity (LA). An arm entry required that all four of the animal's paws be entered into the arm. Increasing the time spent or entries in open arms were considered as components anxiolytic effect (23). An anxiety test was done at the end of the training period on the resting day on all animals without any injection or exercise.

***Statistical analysis***

The data from the anxiety and locomotor activity test was expressed as the mean± standard error of the mean (SEM). These data were analyzed by using one-way analyses of variance (ANOVA). Post-hoc analyses included student-Newman-keuls among multiple groups, and students' T-test between two groups. Statistical significance was set at the $P<0.05$ level.

## Results

***The effect of the exercise activity on anxiety indices and locomotor activity***

Figure 1 showed that after 6 weeks, exercise activity reduced both anxiety indices significantly (%OAT and %OAE) in comparison with the control group ($P<0.05$) (Figure 1, A). But it failed to affect locomotor activity (Figure1, B). This shows the anxiolytic effect of exercise activity.

***The effect of chronic administration of cMgO and MgO NP on anxiety indices and locomotor activity***

The data in figure 2 shows that chronic administration of MgO NP for 6 weeks caused a significant increase in %OAT in comparison with the control group and cMgO ($P<0.001$), while it didn't affect %OAE or the locomotor activity (figure 2, A and B). Chronic injection of cMgO for 6 weeks didn't change any anxiety index or locomotor activity.

***The effect of chronic administration of cMgO and MgO NP during exercise activity on anxiety indexes and locomotor activity***

Chronic injection of cMgO or MgO NP during exercise activity had no effect on the anxiolytic effect of the exercise activity, and made no changes in anxiety indices or the locomotor activity in comparison with the exercise groups (Figure 3A and B).

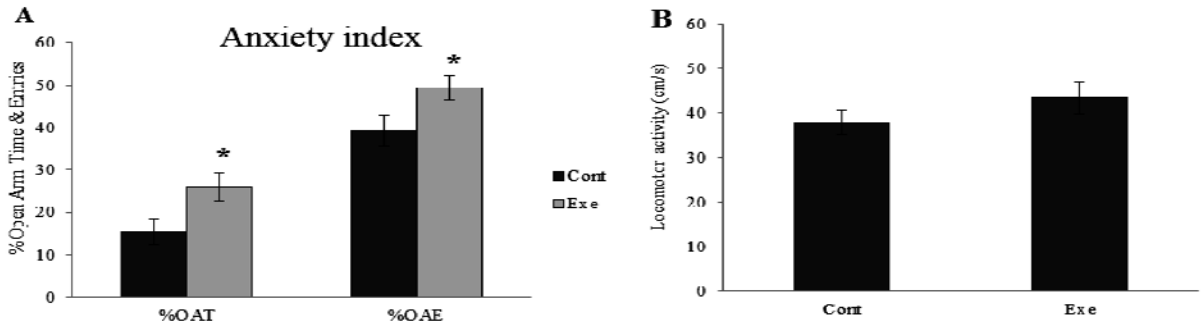


**Figure 1.** The effect of exercise activities on anxiety indices and locomotor activity: each bar shows mean± SEM, *P<0.05 significant differences in comparison with control group (Cont), Exe= exercised group. Student t-test was used for comparison between groups.

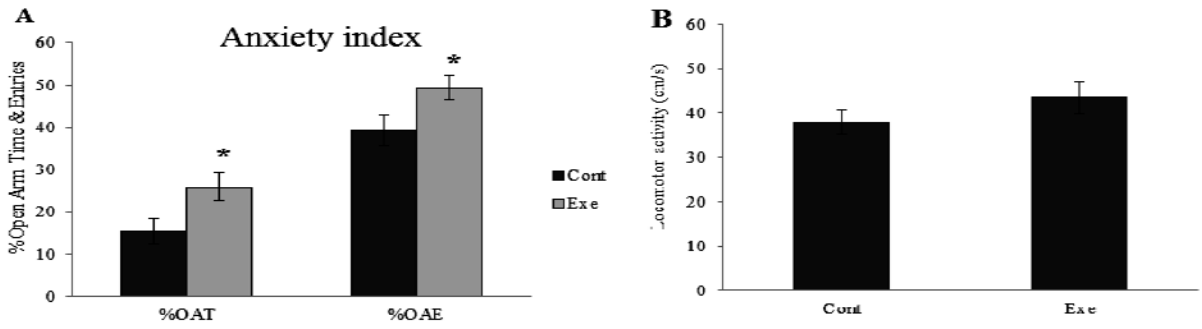


**Figure 2.** The effect of chronic administration of cMgO and MgO NP on anxiety indices and locomotor activity: each bar shows mean± SEM, **P<0.01 in comparison with control group (Cont), ++P<0.01 in comparison with cMgO group.

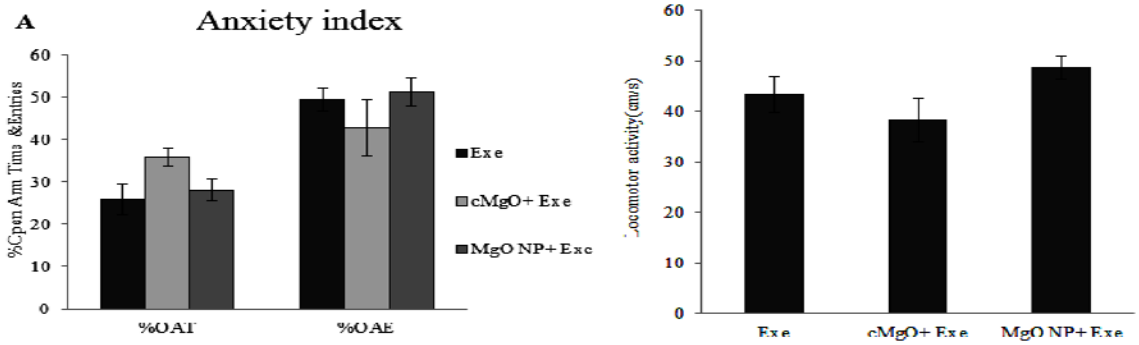


**Figure 3.** The effect of chronic administration of cMgO and MgO NP during exercise activity on anxiety indices and locomotor activity: each bar shows mean± SEM, Exe=exercised group. There were no differences among groups.

## Discussion

Exercise activities cause beneficial effects on stress reduction, anxiety, and depression-like behaviors (12, 24). Several clinical studies have suggested an anxiolytic effect of regular exercise on humans, and some studies have shown that exercise activities are associated with reduced anxiety across several animal types (11, 25). These results supported our findings that an exercise activity after 6 weeks reduced anxiety without changing locomotor activity (Figure 1).

Exercise activities affect many metabolic and physiologic processes, and the direct and indirect involvement of a variety of neurotransmitter and hormonal systems, including serotonin, atrial natriuretic peptide (ANP), and GABA. Every one of these processes can be associated to the anxiolytic effect induced by exercise activities (25-27).

Exercise activities increase the concentrations of atrial natriuretic peptide (ANP). Because ANP has an anxiolytic activity, this hormone might contribute to the anxiolytic effects of aerobic exercise (26). In addition, it has been demonstrated that the endorphin released in the brain of athletes can help reduce their stress and anxiety (28). Our findings also showed that MgO NP reduced anxiety notably after 6 weeks in comparison with the control group and the conventional MgO, both of which had no effect on locomotor activity (figure 2 A and B).

Previous studies have shown that the depletion of Mg leads to an increase in depression and anxiety-related behaviors in mice, while Mg supplementation lessens the anxiety-related behaviors of mice (2, 28). Mg has been proved to modulate both glutamatergic neurotransmission (via a voltage-dependent block of NMDA receptors) and GABAergic neuro-transmission (5-7). It has been demonstrated that increasing glutamate and GABA levels in brain respectively increases and decreases anxiety in animals (2, 23,30). In addition, Mg has been proved to control the activity of the HPA axis, which is considered to be the main stress response system (3). Some studies demonstrated that, since Mg can block this receptor, the blockers of NMDA induce anxiolytic effect (31). It was also noted that the conventional forms of MgO had no effect on anxiety-related behaviors. In fact MgO NP reduced anxiety, while conventional MgO was ineffective. This may be related to the nanoparticles properties and their ability to affect many neurochemical pathways with equal dose (32, 33). Increased surface area of nanoparticles can increase reactive groups more than their conventional form and increase their reactivity (32, 33). Following the injection of nanoparticles and conventional forms of MgO supplements during training made no changes in the anxiolytic effect of exercise activity (Figure 3A and B).

Exhaustive exercise changed the ionized Mg concentration in the extracellular and intracellular compartments. Exercise activity increased the lactate level in athletes, and lactate reduced magnesium level in the body (34). In this study MgO NP and exercise, separately, reduced anxiety, while when applied together, the effect was not better. This may be the anxiolytic effect of MgO NP and exercise activity modulated through common mechanisms like GABAergic system (2).

Another way is that both exercise or magnesium supplement miss the anxiolytic effect of each another. Exercise activity increases lactate and this reduces the level of magnesium in serum, which may reduce the effect of magnesium released from Nano MgO on anxiety-related behaviors. Or Magnesium supplement may act as a compensatory agent for hypomagnesaemia induced by exercise activities (35).

## Conclusion

These findings prove that the chronic administration of MgO NP and exercise activities for 6 weeks reduce anxiety, whereas the anxiolytic effect of them do not increase, when induced at the same time. This interaction is probably related to the common mechanisms involved in anxiety processes, or the reducing effect of one in the presence of the other. To consolidate these findings, more studies need to be done.

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