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Original Article

Magnesium oxide nanoparticles reduce anxiety induced by morphine withdrawal in adult male mice

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Abstract

Introduction: Our previous study has showed that chronic administration of magnesium oxide nanoparticles (MgO NP) can reduce anxiety in adult male rat. In this study the effects of MgO NP on anxiety induced by morphine withdrawal were investigated in adult male mice.

Methods: Adult male NMRI mice (weighing 27 ± 3 g) divided into groups: control, receiving intraperitoneal (i.p.) injection of MgO NP (1, 2.5, 5 mg/kg), morphine withdrawal groups that receiving saline or MgO NP (2.5, 5 &10 mg/kg) as acute (a single injection at the test day) and chronic (co-injected with morphine for 4 days). To develop morphine dependency, increasing doses of morphine (20, 40, 80 mg/kg(injected subcutaneously for 4 days. Mice received a final morphine injection (40 mg/kg) 3 hours prior to naloxone (5 mg/kg (i.p.) on the day of testing (day 4). In addicted groups, after naloxone injection, morphine withdrawal signs were evaluated. In all groups, anxiety like behavior was assessed by the elevated plus maze apparatus.

Results: MgO NP (2.5 & 5 mg/kg) reduced anxiety like behavior ($P<0.05$). Acute and chronic MgO NP injections (5&10 mg/kg) could significantly improve/alleviate anxiety like behavior ($p<0.05$ & $p<0.01$ respectively) and reduce locomotor activity ($p<0.05$, acute; $p<0.05$, & $p<0.01$, chronic), rearing, climbing and weight loss in morphine withdrawn mice.

Conclusion: Due to the positive effect of MgO NP on anxiety like behavior and morphine withdrawal signs and symptoms, this nanoparticle can be a potential candidate for reducing the side effects of chronic usage of morphine and morphine withdrawal.

Keywords:
Anxiety;
Nanoparticles;
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Introduction

Opioid abuse and dependence is a serious threat to the public health in all over of the world and many investigators have studied systems or drugs which could play a role to alleviate morphine withdrawal sings (Habibi-Asl et al., 2015b; Hajhashemi et al., 2004; Kheiry et al., 2015).

Systemic or central administration of opioid receptor agonist, like morphine, induces anxiolytic-like effects in rats on the elevated plus-maze (EPM) test (Torabi et al., 2014). But chronic usage of morphine and its withdrawal in addicted animal leads to the appearance of emotional problems, such as extreme anxiety, major depression dysphoria, craving,

insomnia and bipolar disorder rather than several physical disturbances (Castilho et al., 2008; Miladi-Gorji et al., 2012; Liu et al., 2013).

Anxiety disorder is one of the morphine withdrawal sings and it has been shown that morphine withdrawal results in significant increases in anxiety-like behaviors in animals at the EPM test (Miladi-Gorji et al., 2012).

Current available treatments for opioid dependence, such as methadone and buprenorphine, possess their own abuse liability and are not fully effective at alleviating withdrawal sings (Gamage et al., 2015). Thus new pharmacotherapies that lack abuse potential are needed to alleviate opioid withdrawal sings. On the other hands there are reports showing that pretreatment with magnesium could prevent the development of morphine tolerance and withdrawal symptoms (Habibi-Asl et al., 2009; Habibi-Asl et al., 2015b; Sofiabadi et al., 2010). But the efficacy of conventional forms of magnesium supplements such as magnesium sulfate in improving morphine withdrawal sings needs to be increased by combining with other agents or by applying higher doses (Sofiabadi et al., 2010; Habibi-Asl et al., 2005; Habibi-Asl et al., 2015a).

Magnesium has low ability to cross blood brain barrier (BBB) and intravenous injection of magnesium leads to little increase in levels of magnesium in cerebrospinal fluid (Slutsky et al., 2010; Vural et al., 2010)

Therefore peripheral administration of this element, because of the limitations in crossing BBB, is a fundamental obstacle for the treatment of central nervous system disorders by magnesium supplements.

Recent rapid developments of nanotechnology has provided a basis for usage of nanoparticles in biomedical and industrial applications in human health and environment (Kesmati et al 2014a; Gao 2016). Nano-drugs have higher permeabilityand could therefore cross the BBB with much more efficacy thanconventional forms (Gao 2016). In our previous studies, we have shown that some forms of metal oxide nanoparticles including zinc oxide (ZnO) and magnesium oxide (MgO) nanoparticles, as novel drugs, could affect some physiological behaviors in animals (Jahangiri et al , 2013; Kesmati et al., 2014a; Kesmati et al., 2016; Torabi et al , 2014).

Acute or chronic application of these nanoparticles, show anxiolytic, analgesic and anti-inflammatory effects as well as improved memory formation in animal models (Jahangiri et al., 2013; Kesmati et al., 2014a; Kesmati et al., 2016). There is also evidences that these metal oxide nanoparticles can exert toxic effects in biological systems, and so comprehensive studies are required to evaluate the safety of the use of these novel drugs in the treatment of central nervous system disorders (Shaikh et al., 2015; Mahmoud et al., 2016). Even though some of the physiological effects of MgO nanoparticles as a new source of magnesium has been evaluated in animal models, but its efficacy on anxiety induced by morphine withdrawal is not studied yet. In this study, we investigated the effect of acute and chronic administration of MgO nanoparticles on anxiety like behavior induced by withdrawal of morphine in adult male mice.

Materials and methods

Animals and drugs

Adult male mice (weighting 27±3 gr) were purchased from animal house of Joundi Shapor University of Medical Sciences in Ahvaz, Iran. Animals were kept in room condition with 12/12 h dark/light cycle and temperature 23±1 ºC and had free access to food and tape water. MgO nanoparticles (NP) (lolitech Co, Germany, particle size <50 nm) were dispensed in saline 0.9% by ultrasonic bath (Parsnahand Co, Iran) for 20 minutes and shaken for 1 minute before each injection. Morphine sulphate (Temad Co, Iran) and naloxone hydrochloride (Sigma Co, Germany) were dissolved in saline 0.9%.

Mice were randomly divided into two main groups :1) healthy mice, including control group (receiving saline 0.9%) and groups receiving intraperitoneally (i.p.) injection of MgO NP (1, 2.5, 5mg/ kg) 30 minutes before elevated plus maze test (acute), 2) morphine withdrawn mice,receiving saline 0.9% or receiving i.p. injection of MgO NP (2.5, 5, 10 mg / kg) (a single injection 30 minutes before naloxone on the test day (as acute) and four injections during morphine administration starting on first and continuing to forth day (as chronic) (Jahangiri et al., 2013; Kesmati et al., 2014b). Number of animals in each group were 8 (N=8). All procedures were carried out in accordance with institutional guidelines for animal care and use

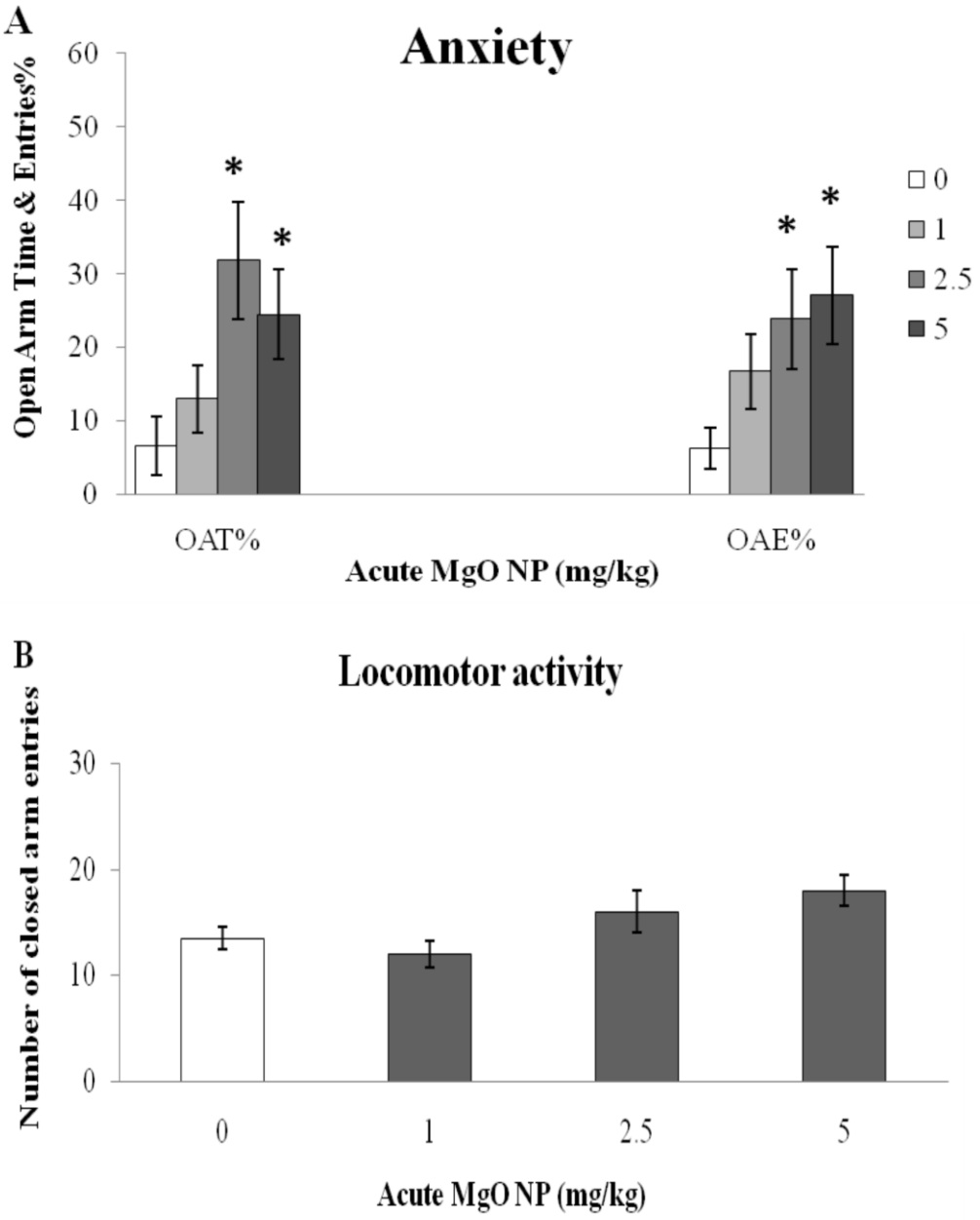

Fig.1. The effect of acute injection of MgO NP (1, 2.5 & 5 mg/kg) on anxiety indexes and locomotor activity. *P<0.5 shows significant differences between control (0) and MgO NP receiving groups. All data are expressed as mean ± SEM, (N=8).

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Morphine dependence and evaluation of morphine withdrawal sings

In order to induce dependence in mice, morphine was administered subcutaneously 3 times per day at 9 a.m. (20 mg/kg), 1 p.m. (40mg/kg) and 5 p.m. (80 mg/kg) for 3 consecutive days. Three hours after the injection of the morphine 40mg/kg on the fourth day, naloxone (5 mg/kg, i.p.), the selective antagonist of opioid receptors, was injected into the addicted animals. To precipitate withdrawal syndrome, mice were immediately placed individually in a plexiglass box and numbers of rearing and climbing were recorded during a 30-min period (Kesmati et al., 2009) and then the anxiety like behaviors were evaluated by elevated plus maze. Also weight loss during 24 hours after morphine withdrawal was measured in all addicted mice.

Elevated plus maze (EPM)

The Plexiglass elevated plus maze consisted of two opposite open arms (20cm ×5 cm) and two closed arms (20 cm×5 cm with 15cm walls) in the shape of a cross that connected by a central square (5×5 cm) was made in Shahid Chamran University in Ahvaz.

The maze was elevated 50 cm above the ground and animals were placed in the center square facing an open arm and allowed to explore the maze for five minutes while their behavior was being recorded by the camera and analyzed. The behaviors examined included: percentage of open arm time (open/(open+closed); %OAT) and percentage of open arm entries (open/(open+closed); %OAE). Number of entries in close arms in 5 minutes was calculated as locomotor activity. An arm entry required that all four of the animal's paws entered into the arm. Increasing the time spent in or entries to open arms were considered as components anxiolytic effect.

Statistical analysis

Data were expressed as mean ± SEM. Student's t-test was used for comparison of the means of unpaired data. For multiple comparisons between groups we used one way ANOVA and Tukey post hoc test. The analyses were performed by using Instate 3 software. Differences with a $P<0.05$ between experimental groups at each point were considered statistically significant.

Results

Effect of acute injection of MgO NP (1, 2.5 & 5 mg/kg) on anxiety indexes and locomotor activity

Results on figure 1 show that acute injection of MgO NP 2.5 and 5 mg/kg increased OAT % and OAE% ($P<0.05$) without changes on locomotor activity in EPM test. This shows anxiolytic effect of acute administration of MgO NP.

Effect of morphine withdrawal on anxiety indexes and locomotor activity

Figure 2 showed that morphine withdrawal in

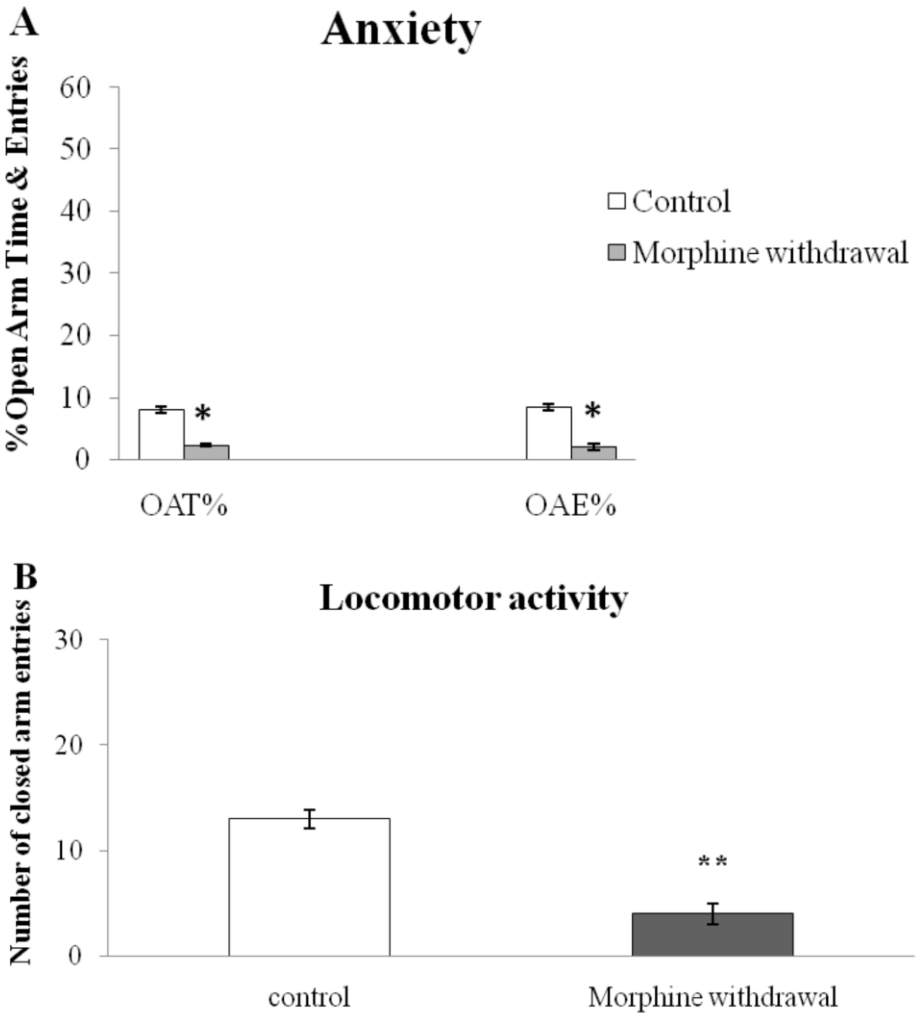

Fig.2. The effect of morphine withdrawal on anxiety indexes and locomotor activity. *P<0.5 and **P<0.01 show significant differences between control and morphine withdrawal group. All data are expressed as mean ± SEM, (N=8).

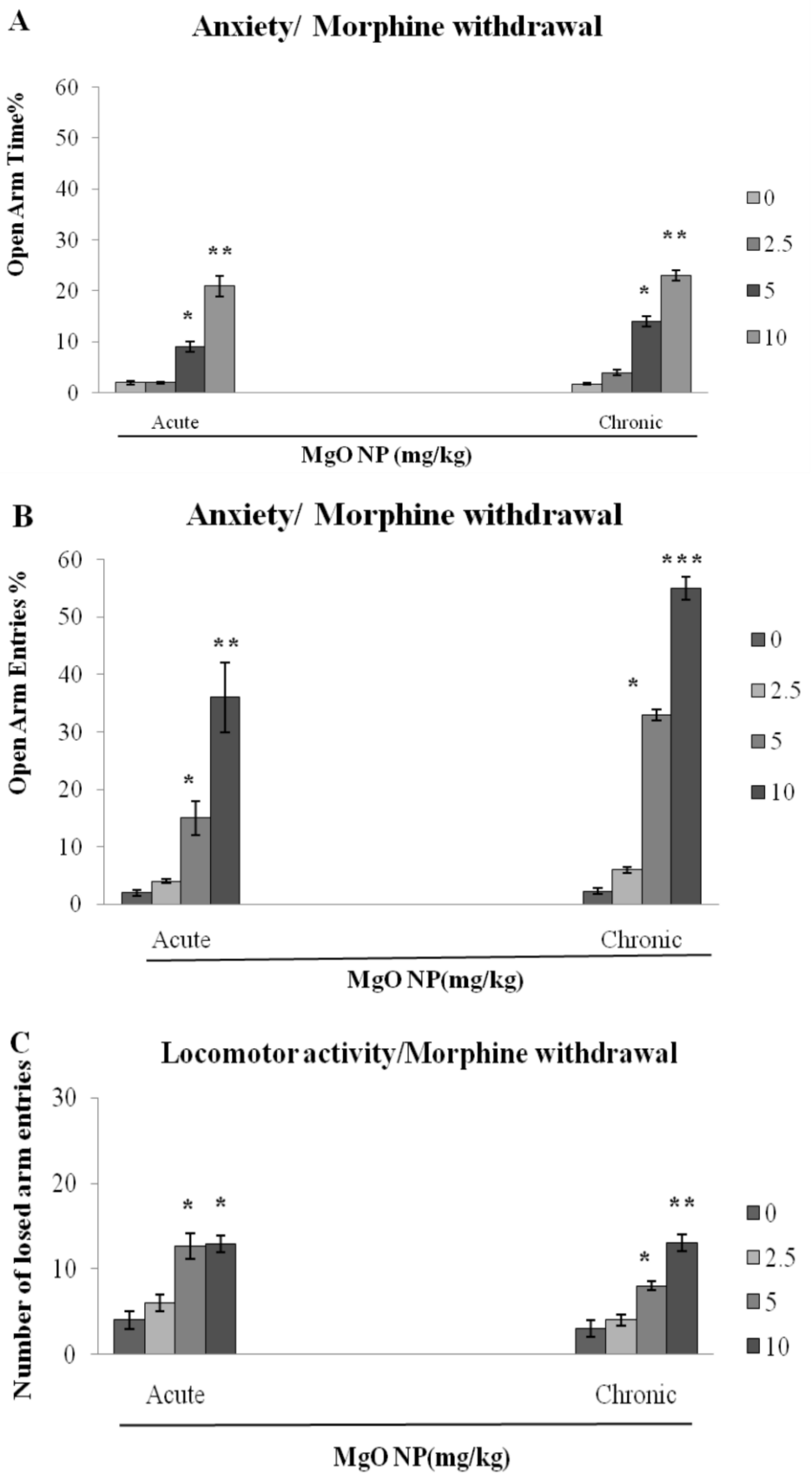

Fig.3. The effects of acute and chronic injection of MgO NP (2.5, 5 & 10 mg/kg) on anxiety indexes and locomotor activity in morphine withdrawn mice. *P<0.05, **P<0.01& ***P<0.001 show significant difference in comparison with control (0) group in each column. All data are expressed as mean ± SEM, (N=8).

Table 1: Effect of acute and chronic injection of MgO NP (2.5, 5 & 10 mg/kg) on morphine withdrawal signs.

		Acute injection			Chronic injection		
	Morphine withdrawal sings	Weight loss (Mean± SEM)	Rearing (Mean± SEM)	Climbing (Mean± SEM)	Weight loss (Mean± SEM)	Rearing (Mean± SEM)	Climbing (Mean± SEM)
Groups(dose)	Control (saline)	2.8±0.4	6.3±1	8.5±1	2.5±0.5	6±0.8	8±1
	MgO NP (2.5 mg/kg)	1.7±0.5	5.5±0.9	8±1	1.5±0.4	3.5±0.3*	3±0.4*
	MgO NP (5 mg/kg)	0.7±0.2	5±0.8	6±0.5*	0.6±0.1	3.2±0.5*	2±0.1*
	MgO NP (10 mg/kg)	0.5±0.1	4.3±1*	4±0.5*	0.4±0.1	3±.0.2**	1.8±0.1**

*p<0.05 and **p<0.01 are in compared with control group in every kind if injection (acute or chronic).

addicted mice decreased OAT% and OAE% (P<0.05) and reduced locomotor activity (P<0.01). This indicates that morphine withdrawal increases anxiety in addicted mice and affects locomotor activity.

Effects of acute and chronic injection of MgO NP (2.5, 5 & 10 mg/kg) on anxiety indexes and locomotor activity in morphine withdrawn mice

Results on figure 3 showed that acute and chronic injections of MgO NP (5 and 10 mg/kg) significantly increased OAT% and OAE% (P<0.05 & P<0.01) in morphine withdrawn groups. These show that the MgO NP administration can alleviate anxiety like behavior resulted from morphine withdrawal. Also MgO NP (5 & 10 mg/kg) can improve locomotor activity in acute (P<0.05) and chronic (P<0.05 &P<0.01) injection (figure 3B). According to these results highest dose (10 mg/kg) and chronic injection of MgO NP was more effective on improving anxiety like behavior and locomotor activity in morphine withdrawn mice.

Effect of acute and chronic injection of MgO NP (2.5, 5 & 10 mg/kg) on morphine withdrawal signs

Table 1 shows that acute injection of MgO NP 5 &10 mg/kg reduced number of rearing and climbing in comparison with control group (P<0.05). Also, chronic injection of MgO NP reduced number of rearing and climbing in a dose dependent manner (p<0.05, at the doses of 2.5 and 5 mg/kg and p<0.01, at the dose of 10 mg/kg). These results showed that chronic injection of MgO NP was more effective than its acute administration on alleviation of morphine withdrawal sings. Mean value of weight loss in all MgO NP receiving groups was lower than the control group even thought was not significant.

Discussion

Our study showed that acute injection of MgO NP in male mice reduces anxiety like behavior in a dose dependent manner (Figure 1). It has been previously shown that magnesium supplementation lessens the anxiety-related behaviors in animals which is consistent with our findings (Poleszak 2008; Laarakker et al,. 2011).

N-methyl-d-aspartate (NMDA)/ glutamate pathway is an important mechanism in anxiolytic effects of magnesium (Młyniec et al., 2014). Magnesium exerts a physiological block of the ion channel on the NMDA receptors, preventing influx of extracellular calcium and activation of secondary neuronal changes (Ghasemi et al., 2010; Laarakker et al., 2011). Some studies suggest the involvement of NMDA receptors in anxiolytic effects of magnesium (Kesmati et al., 2016; Laarakker et al., 2011; Młyniec et al., 2014).

Another most widely accepted mediators playing a central role in the pathophysiology of anxiety disorders is the GABAergic (gamma butyric acid) system which can be also modulated by magnesium (Kumar et al., 2013; Poleszak 2008). In addition, the limbic–hypothalamus–pituitary–adrenocortical (HPA) axis is thought to be influenced by magnesium deficiency (Jacka et al., 2009; Młyniec et al., 2014). These are probably some of the underlying mechanisms of the anxiolytic effect of magnesium released from MgO NP that are also in correlation with our previous study in animals (Kesmati et al., 2014b).

Furthermore, our results showed that morphine withdrawal induced by naloxone 5mg/kg increases anxiety levels and reduces locomotor activity in addicted mice (figure 2). Several studies have demonstrated that opioid withdrawal is associated with anxiety in humans and experimental animals (Castilho et al., 2008; Wen et al., 2014; Vahidi et al., 2015). Interestingly, we proved that acute and chronic injection of MgO NP could reduce anxiety like behavior, locomotor activity impairment and other morphine withdrawal sings (figure 3 & table 1).

As previously described, administration of magnesium can attenuate morphine tolerance and dependency probably through the blockage of the NMDA receptors and its interaction with nitric oxide (NO) or other non-opioid systems (Sofiabadi et al., 2010; Habibi- Asl et al., 2015b).

Non-opioid systems like GABA, NO, NMDA and glutamate receptors play an important role in the development of adverse effects of opioids (Bhalla et al., 2016). While a variety of competitive and non-competitive NMDA receptor antagonists or NO synthase inhibitors were found to suppress the development of morphine tolerance and dependence (Cappendijk et al., 1993; Karami et al., 2014; Toda et al., 2009).

Chronic treatment with opioids leads to the activation and translocation of protein kinas C, which phosphorylates the NMDA receptor-gated calcium channels, and results in the potentiation of NMDA receptors activity (Zhu et al., 2001). Activation of the ionotropic NMDA subtype of glutamate receptors has been shown to be implicated in the development of morphine analgesic tolerance and dependence (Habibi-Asl et al., 2015b). There is evidence indicating that noncompetitive NMDA receptor antagonists like MK-801 inhibit the development of mu receptor-mediated opiate dependence in adult rodents (Barr et al., 2011; Zhu et al., 2001). Therefore, it seems reasonable to suggest that glutamate affecting the NMDA receptors may play a role in modulation of anxiety in mice exposed to the EPM (Faria et al., 2016).

In addition, nitric oxide synthtase (NOS) inhibitors like L-NAME, are also shown to prevent tolerance to morphine and alleviate nicotine withdrawal syndrome in animals, implying an underlying role of NO (Jain et al., 2008; Karami et al., 2011).

Long term administration of opiate impairs the blocking effect of magnesium on NMDA receptor calcium channels and therefore increases intracellular calcium (Habibi- Asl et al., 2005; Habibi-Asl et al., 2015a). Thus, it could be postulated that MgO NP may have a potential role in prevention of anxiety induced by morphine withdrawal through interacting with NMDA receptor.

While it has been shown that higher doses or chronic use of nanoparticles can induce toxicity in animals (Kesmati et al., 2014a; Shaikh et al., 2015), in our study chronic injection of MgO NP at high dose was more effective than its acute injection or lower doses to combat the anxiety like behavior, rearing and climbing resulted from morphine withdrawal. Therefore, MgO NP can be considered as an effective agent in the treatment of anxiety disorder and opioid dependence, though as a new drug more safety and efficacy studies are required.

Conclusion

Our findings showed that acute and chronic administration of MgO NP can reduce anxiety like behavior and other signs of morphine withdrawal in mice. Chronic MgO NP exerted a much strong effect in alleviation of anxiety like behavior and other morphine withdrawal sings. Finding the exact mechanisms involved in the anxiolytic effect of MgO NP and opioid/ non-opioid system activity can be a topic for future studies.

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Conflict of interest

The authors declare no conflict of interest.

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