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ORIGINAL RESEARCH PAPER

Vitamin C potentiate sedative effect of magnesium oxide nanoparticles on anxiety and nociception in the postpartum depression model

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ABSTRACT

Objective(s): Our previous studies have shown that MgO nanoparticles (MgO NPs) could improve anxiety and reduce pain in animals. In this study, the effect of co administration MgO NPs and vitamin C on anxiety like behavior and nociception in postpartum depression (PPD) model were investigated.

Materials and Methods: Female mice (27 ± 3 g) were divided into groups: two control groups, PPD groups: saline or MgO NPs 1, 5, 10 mg/kg (acute/ chronic), vitamin C 5 and 25 mg/kg (acute) alone or co-injected with MgO NPs 5 (acute). For induction of PPD, chronic injection of progesterone for 5 days was used and three days after progesterone withdrawn (PWD), the depression behavior was evaluated by tail suspension test. Elevated plus maze and hot plate tests were used for evaluation of anxiety and pain perception, respectively.

Results: PWD induced anxiety and acute injection of MgO NPs 5 reduced anxiety while chronic injection increased anxiety ($P<0.05$). Acute and chronic injections of MgO NPs 10 increased anxiety ($P<0.05$, $P<0.001$and $P<0.01$). Vitamin C 5 reduced anxiety ($P<0.05$) and co-injection of MgO NPs 5 with vitamin C 25 increased anxiolytic effect ($P<0.01$). Acute injection of vitamin C 25 and co-injection of MgO NPs 5 and vitamin C 5, in comparing with acute MgO NPs 5, increased latency time at the hot plate test ($P<0.05$).

Conclusion: Effects of MgO NPs on anxiety like behavior induced by PWD, is depends on dose and usage duration. Probably usage of antioxidant can improve efficacy of MgO NPs on anxiety reduction and nociception.

Keywords: *Anxiety, Nanoparticles, Nociception, Postpartum depression, Vitamin C*

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INTRODUCTION

Postpartum depression (PPD) is a major kind of depression in women [1]. The onset of depressive symptoms is to the rapid postpartum withdrawal of the ovarian hormones estradiol and progesterone that occurs during the first five days following childbirth [1-3]. Depression is often accompanied by enhanced anxiety, in animal models chronic exposure to progesterone, followed by its withdrawal increases anxiety related behavior [4, 5].

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Magnesium is one of the most essential minerals in the human body physiological function [6, 7]. The huge amount of magnesium absorbed from the placenta by the fetus and the mother loss of magnesium that is hypothesized to be a contributing factor in the development of PPD [7]. Also Magnesium deficiency can increase anxiety like behaviors, while treatment with magnesium supplements exerts antidepressant and anxiolytic effects by acting on neurotransmitters and hormonal pathways [5, 8]. Scientists have shown that the usage of magnesium supplements can reduce PPD symptoms and anxiety like behaviors resulted from it in animals [4, 5]. It has been shown that the usage of magnesium

supplements can significantly reduce the pain in animals and preoperative intravenous magnesium sulfate infusion decrease post-operation pain and requirement of analgesia in cesarean section in women [9-11].

By development of nano medicine, investigated the effects of metal oxide nanoparticles in some models of animal behavior for replacement with older forms of metal oxide supplements growth up quickly [12-19]. Nano drugs based on metal oxide have ability in crossing of biological barrier in the body [14, 15, 17].

Efficacy of MgO NPs as a new form of magnesium supplement in central nervous system (CNS) disorders, especially on anxiety related behaviors and pain perception investigated in some of our previous studies [12, 16,17]. Our Studies showed that acute and chronic injection of MgO nanoparticles can reduce anxiety like behaviors and acute or chronic pain perception in animals [12, 16, 18].

There are evidences that nanoparticles can cause neurotoxicity by producing of oxidative stress and has been shown that MgO NPs can increase reactive oxygen species gene (ROS) expression and make toxicity in biological systems [19-21]. Several nanoparticles exhibit detrimental effects on female reproductive system and fetal development that these adverse effects are related to nanoparticle properties, dose, exposure route or even animal species [22- 24]. Nanoparticles have the ability to transfer from mother to offspring and accumulate in the most maternal organs and embryos [22- 24].

On the other hands it has been demonstrated that ascorbic acid as an antioxidant in the central nervous system plays an important therapeutic role in anxiety like behavior and nociception in humans and animals [25, 27]. Our previous study indicated that vitamin C could increase ZnO NPs efficacy in reduction of morphine dependence in adult male mice [28]. According to these studies, assess the usage of nanoparticles as a source of metals in female at the duration of pregnancy and after child birth need to more investigations. Also, introduce of MgO NPs as a new supplement of magnesium for treatment of anxiety like behaviors or pain perception in clinical usage such as the PPD need for significant data.

In this study, we investigated the effect of acute and chronic administration of MgO NPs on anxiety like behaviors and acute somatic pain perception at present and absent of vitamin C as an antioxidant in a PPD model of female mice.

MATERIALS AND METHODS

Animals

In this experimental study the subjects were female NMRI mice (weighing 27 ± 3 gr) purchased from the animal house of the Joundi Shapor University of Medical Sciences (Ahvaz), Iran. Mice were accommodated for more than a week in a room at 24 ± 1°C, with controlled 12/12 hours light-dark cycles. They were housed in polypropylene cages (6 per cage). Food and drinking water were freely available except during the test periods. In each experiment 6-8 animals were used. Each animal was used once only and experiments undertaken during the light phase. All procedures were carried out in accordance with the institutional guidelines for animal care and use at the Shahid Chamran University of Ahvaz, Iran. All behavioral testing took place in a dimly lit room. All mice were allowed to adapt to their caging environment for at least two weeks prior to the induction of PPD. Animals adapted to the testing room over a one hour period prior to testing.

Drugs and treatments

MgO NPs (particle size < 50 nm, Lolitec Co., Germany) at the dose of 1, 5 and 10 mg/kg dispersed in saline 0.9% by ultrasonic bath (Pars Nahand Co., Iran) for 20 minutes and shaken for one minute before of each injection, in chronic injection animals received daily injection of MgO NPs for 8 days, in acute injection animals received MgO NPs thirty minutes before tests. XRD pattern was recorded using a Philips Analytical X-Ray diffractometer operated at 40 kV and 30 mA by means of the Cu Ká radiation. Fig. 1 shows the intensity of the reûection in the XRD pattern is related to MgO NPs (JCPDS card no. 4–829). Vitamin C (5 and25 mg/kg) (Sigma, St. Louis, MO, USA) was dissolved in saline 0.9% and was injected in acute (30 minutes before each test) and chronic (daily for 8 days). Progesterone (5 mg/kg) (Sigma, St. Louis, MO, USA) was dissolved in sesame oil (vehicle) (Barij Esans Co, Iran) (2.5 mg/ml) and was injected. To induce PPD, all animals received daily progesterone for five days, after which progesterone was withdrawn for three days. In a preliminary study to confirm the effect of progesterone withdrawal (PWD) two groups which received saline and sesame oil instead of

progesterone served as the control groups. Drugs were administered in a volume of 2 ml/kg and all drugs injected intraperitoneally (i.p.). In all experiments the interval time between injections and behavioral tests was thirty minutes.

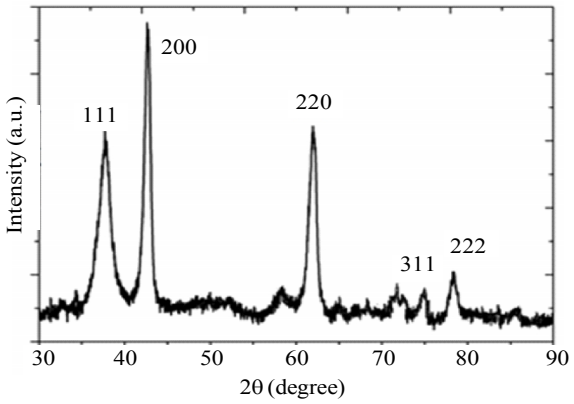

Fig. 1. XRD patterns of MgO NPs. Diffraction angle is between 30° and 90°

Behavioral experiments

Tail suspension test

The tail suspension test (TST) was performed just for validation, PPD model induced by progesterone withdrawal. The mice were individually suspended on the hook of the tail suspension test box, 60 cm above the surface of the table with an adhesive tape placed 1 cm away from the tip of the tail. After one minute acclimatization, immobility duration was recorded for five minutes from side view using small ûre-wire cameras. Mice were considered immobile only when they hung passively and were completely motionless [29].

Elevated plus maze

The wooden plus maze (made in Shahid Chamran University of Ahvaz, Iran) consisted of two open arms (30×5 cm), and two closed arms of the same size but with 15 cm high end and side walls. The arms were connected by a central 5×5 cm area and there were no walls on the open arms. The height of the elevated plus maze (EPM) above the floor was 50 cm. Rats were placed in the center of the EPM with their head facing an open arm and left undisturbed for 5 minutes. Mice were then removed and returned to their home cages.

The experimental sessions were recorded by camera. A mouse was considered to be on the central platform when at least two paws were on it and on an arm whenever all four paws were on it. Percent of time spent in open arms [open arm time OAT%: (time in open arm/time in open + closed arm) ×100] and percent of open arm entries [open arm entries OAE% :(number of open arm entries/ number of open + closed arm en tries) ×100] were used as a measure of anxiety [14].

The number of close arm entries in 5 minutes was used as a measure of locomotor activity

Hot plate test

For measurement of acute somatic pain perception, animals were placed on a hot plate apparatus maintained at 53±1 ºC and the latency to lick the hind foot was recorded as pain index. A 120 seconds cut-off point was used to prevent tissue damage if no response occurred.

Statistical analysis

Data were expressed as mean ± SEM. Student's t test was used for comparison of the means of unpaired data. ANOVA was used for multiple comparisons between groups and Tukey post hoc test was performed using Instate 3 software.

P< 0.05 was considered to be statistically signiucant.

RESULTS

Tail suspension test (TST): The results of table 1 show that daily injections of progesterone for five days that after which progesterone was withdrawn for three days induced depression in female mice by increasing immobility time in comparison with the group receiving sesame oil and/ or saline alone (***P<0.001).

This just shows the validation of postpartum depression induced by progesterone withdrawal.

Table 1. Comparison between Immobility time in different groups at postpartum depression test. ***P<0.001 show significant difference between Progesterone (PWD)/ saline (acute) group and vehicle/ saline group. N=6-8

Groups	Immobility time
Saline/ Saline	42.87±7.4
Vehicle/ Saline	50.5±14.26
Progesterone(PWD)/ Saline(acute)	111.14±6***
Progesterone(PWD)/ Saline(chronic)	100.28±9.6

Elevated plus maze (EPM) test

Results in Fig. 2 (A) show an anxiety like behaviors, increased in PWD (or PPD model) group (A) without any changes in locomotor activity (B) through a reduction in OAT % and OAE % (*P<0.05) in comparison with vehicle or saline group.

Data in Fig. 3 (A) show that acute administration of MgO NPs 5 mg/kg has induced significant anxiolytic effect, Through increasing OAT% and/or OAE% (*P<0.05) in PWD (or PPD model) group, but MgO NPs 10 mg/kg showed an anxiogenic effect with reduction of OAT% and OAE% (**P<0.01, ***P<0.001 respectively).

Chronic injection of MgO NPs in 1 mg/kg was ineffective while in doses of 5 and 10 mg/kg potentiated anxiety induced in PPD groups with reduction of OAT% and OAE% (P<0.05). Comparison between acute and chronic injection of MgO NPs shows that by rising dose and duration of administration cause adverse effects, so that chronic injection of MgO NPs 5mg/kg significantly reduced anxiety indexes OAT % and OAE % (#P<0.05) in compared with acute injection of it, but dose 10 mg/kg showed an anxiogenic effect in both acute and chronic injection (Fig. 3 A).

Acute or chronic injection of MgO NPs in all doses didn't change locomotor activity (Fig. 3 B). Fig. 4 (A) shows that even though acute injection of every dose of vitamin C 5 and 25 mg/kg could improve anxiety induced by PWD, but just vitamin C 5mg/kg significantly increased OAT % and the OAE% (P<0.05) (Fig. 4 A) without any changes in locomotor activity (Fig. 4 B).

At the follow we selected acute MgO NPs 5mg/kg (as the most effective dose for reduction of anxiety induced by PWD) for co injections with acute vitamin C 5 and 25 mg/kg.

Data in Fig 5 (A) showed that acute co injection of MgO NPs 5 mg/kg and vitamin C 25 mg/kg significantly increased OAT% (P<0.01) in comparison with MgO NPs 5mg/kg alone (Fig. 5 A). These results showed that vitamin C could potentiate the anxiolytic effect of MgO NPs without any changes in locomotor activity (Fig. 5 B).

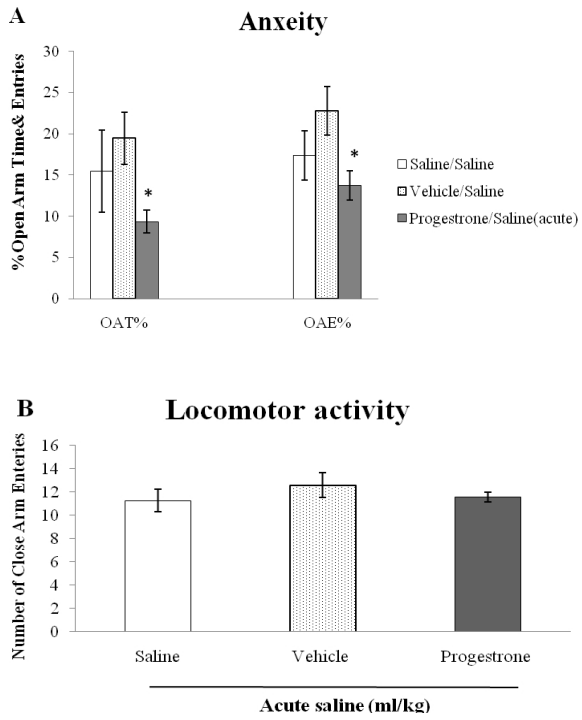

Fig. 2. The effect of progesterone withdrawn on anxiety parameters (A) and locomotor activity (B). Each bar shows mean± SEM. At the Fig. 2A: *P<0.05 shows significant difference between Saline (acute)/ Progesterone and Vehicle/ Saline groups in OAT% and OAE%. N=6-8

Hot plate test

Results in Fig. 6 (A) show that, PWD could not affect the acute pain perception in the hot plate test also acute or chronic treatment by MgO NPs in all groups could not significantly change latency time. But in PWD mice, acute usage of vitamin C 25 mg/kg could increase the latency time in comparison with control group (*P<0.05).

also, acute co-injection of MgO NPs 5 with vitamin C increased latency time in both of two groups and it was significant in Comparison to vitamin C 5mg/kg (+P<0.05) (Fig. 6 B).

These results show that vitamin C can be effective in the reduction of acute pain perception in PWD female mice.

DISCUSSION

The results in the Fig. 2A showed that progesterone withdrawn increased anxiety in female mice. This result confirms some studies that have shown during the postpartum, anxiety increased that may result from the rapid withdrawal of ovarian hormones, especially abrupt decreases in progesterone [2, 3, 5].

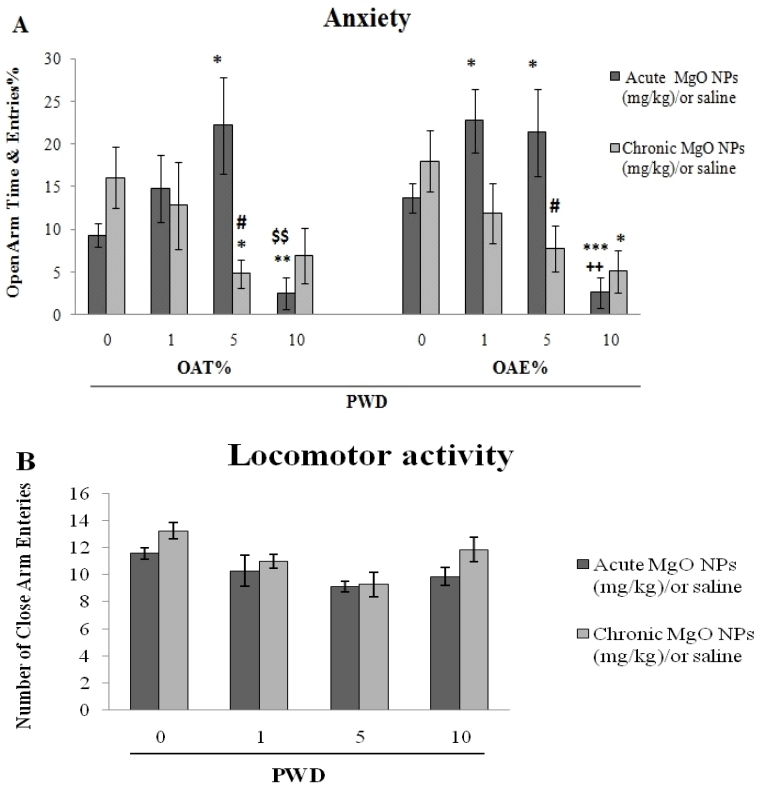

Fig. 3. The effect of acute and chronic administration of MgO NPs on anxiety parameters (A) and locomotor activity (B) at PWD groups. Each bar shows mean± SEM. At the Fig. 3A, in each column: *P<0.05, **p<0.01 and ***P<0.001, show significant difference in compared with PWD /saline(0)(acute or chronic) groups(in OAT% & OAE%), # P<0.01 shows significant difference in comparison with PWD/ acute MgO NPs 5mg/kg in OAT% &OAE%, ++P<0.01 shows significant difference in comparison with PWD/ acute MgO NPs 1mg/kg in OAE%, $$P<0.01 shows significant difference in comparison with PWD/ acute MgO NPs 5mg/kg in OAT%. N=6-8

GABA is a major inhibitory neurotransmitter in the central nervous system and one of the most important neurotransmitters in anxiety behaviors [30, 31]. Progesterone metabolites, act on GABA receptors in the brain, and treatment with progesterone produces sedative-like effects by enhancing GABA neurotran- smission that lead to reduce anxiety [5, 31].

In the other results, we showed that only acute injection of MgO NPs improves anxiety induced PPD model (Fig. 3A) that this effect is reversed at chronic administration. It was for the first time that we have shown MgO NPs can be effective on anxiety in PWD model in female mice. There are various evidences that have shown magnesium supplement can improve anxiety and depression in animals by acting on hormonal pathways and neurotransmitters like λ-. Aminobutyric acid (GABA) and glutamate [7, 8, 12]. Magnesium modulates glutamatergic neuro-transmission (via a voltage-dependent block of N-Methyl-D-Aspartate receptor, (NMDA)) and reduces the effect of glutamate as an excitatory amino acid in anxiety like behaviors [32, 33].

In this study it has shown that acute and chronic injection of the highest dose of MgO NPs (10 mg/kg) and chronic administration of 5 mg/kg, potentiated anxiety induced by PPD model (Fig. 3A). Therefore, it should be considered the time and usage dose of MgO NPs in clinical condition. It has been identified that in high density by increasing the percentage of aggregation and accumulation of metal nano-particles, bioavailability of free ions from them reduced [14]. Studies indicated that nanoparticles have high ability to make free radical species that

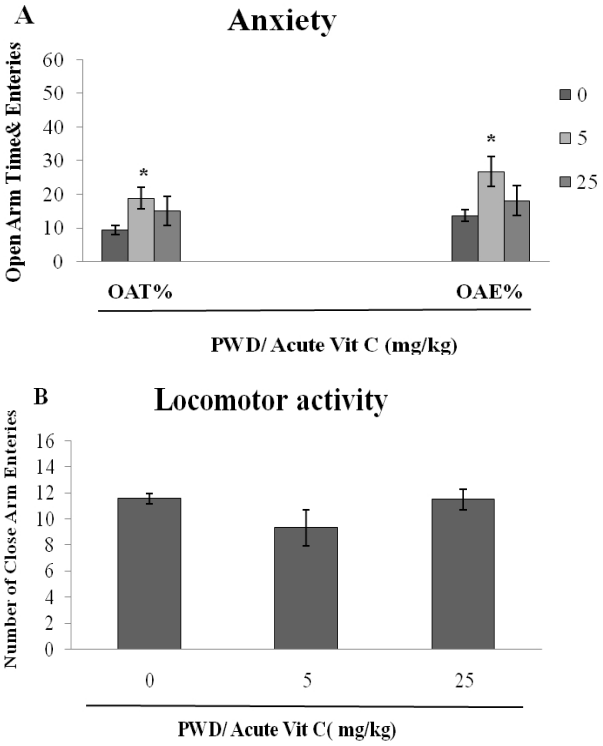

Fig.4: The effect of Vitamin C on anxiety parameters (A) and locomotor activity (B) at PWD groups. Each bar shows mean± SEM. In each column: *P<0.05, shows significant difference in comparison with saline group (0) (in OAT% & OAE). Vit= Vitamin, N=6-8

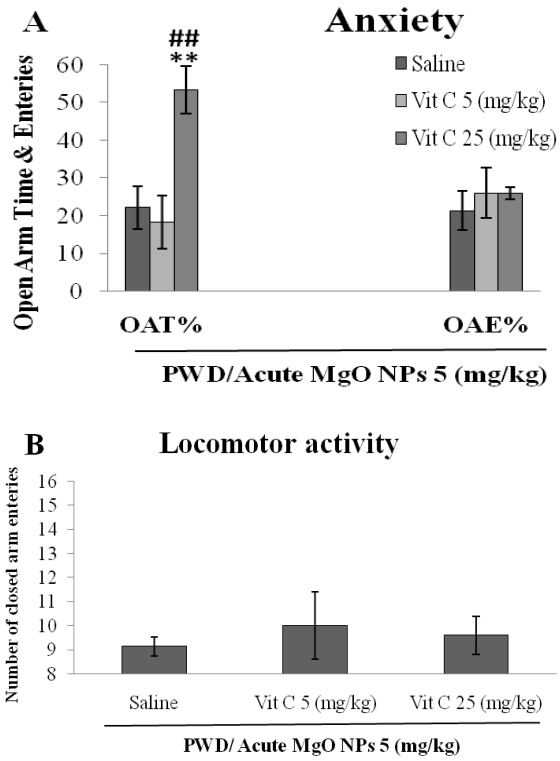

Fig. 5. The effect of acute co administration of MgO NPs and Vitamin C on anxiety parameters (A) and locomotor activity (B) at PWD groups. Each bar shows mean± SEM. In each column: **P<0.01, shows significant difference in comparison with control group (in OAT) and ##P<0.01 shows significant difference in comparison with vitamin C 5mg/kg (in OAT %). Vit= Vitamin, N=6-8

these productions can have undesirable effects on many tissues including the brain [19, 23]. These effects of the highest dose of MgO NPs on anxiety may be related to the accumulation of nanoparticles in central or peripheral organs of animal and nanoparticles toxicity [14]. So the usage of MgO NPs in PWD female mice depends on dose and duration of treatment. On the other hand, acute injection of vitamin C could improve anxiety in PWD mice and increased the anxiolytic effect of MgO NPs (Figs 4 and 5 A). Ascorbic acid is considered an important neuroprotective agent that works as an antioxidant and scavenges reactive oxygen species in central nervous system [26]. Ascorbic acid modulates the activity of glutamate receptors like NMDA and GABA receptors and has an inhibitory role on NMDA receptor functioning that protects neurons against glutamate excitotoxicity [34-36]. Also, vitamin C treatments could reduce acute pain perception and co-injection of MgO NPs with vitamin C could significantly increase the analgesia time in the PPD animal model (Fig 6 B). Thus the analgesic effect as well as anti-anxiety effect can be seen with the co administration of vitamin C and MgO NPs. There is a correlation between pain and anxiety pathways in some area of the brain and some neurotransmitters such as glutamate and GABA are involved in these two phenomena [37, 38].

As previously described, probably vitamin C by direct or indirect pathways can modulate GABAergic and glutamatergic pathways or acting as an antioxidant agent to increase anxiolytic and analgesic effects of MgO NPs.

CONCLUSION

According to the results of this study, acute and low dose administration of MgO NPs can be a

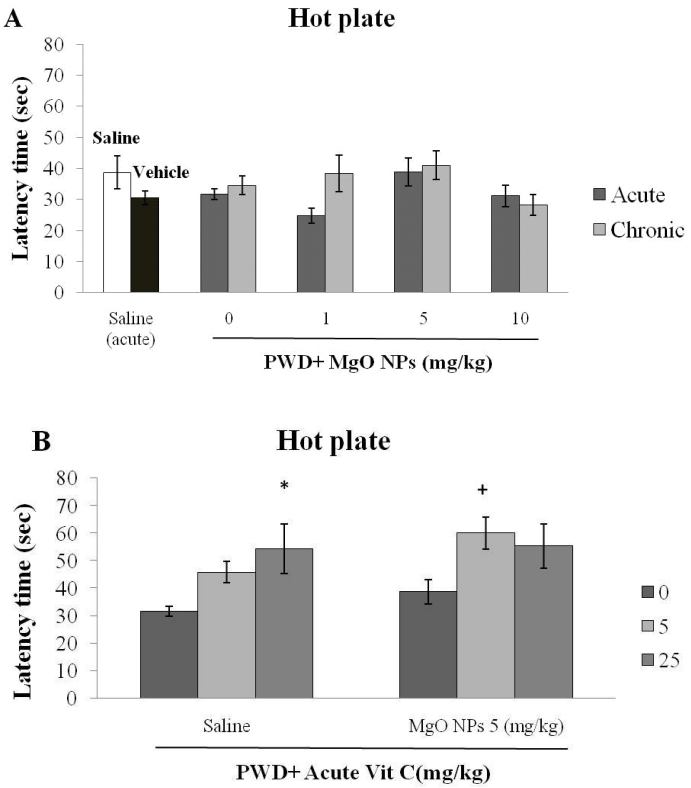

Fig. 6. The effect of acute and chronic injection of MgO NPs (A) alone and with vitamin C (B) on nociception in hot plate test. Each bar shows mean± SEM. *P<0.05 show significant difference in comparison with Saline/ PWD+ Acute Vit C 0 group, +P<0.05 show significant difference in comparison with MgO NPs 5(mg/kg)/ PWD+ Acute Vit C 0 group. Vit= Vitamin, N=6-8

suitable supplement for improving of anxiety induced by PWD in female mice. Probably vitamin C as an antioxidant can be a suitable adjustable component to increasing anxiolytic and analgesic effects of MgO NPs in animal models. Finding the exact mechanisms of vitamin C and MgO NPs functions in above physiological behaviors need for more investigation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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