Nanomed Res J 9(2):164-171, Spring 2024

RESEARCH ARTICLE

Comparative effects of MgO nanoparticles and conventional MgO on anxiety and serum biochemical parameters in sleep deprivation model of rat

Mahnaz Kesmati[1], Ebrahim Nabaee[1], Ali Shahriari[2], Mozhgan Torabi[1*]

[1] Department of Biology, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran

[2] Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

ARTICLE INFO

Article History:
Received 03 May 2024
Accepted 19 Jun 2024
Published 01 Jul 2024

Keywords:
Sleep deprivation
Magnesium Oxide
Anxiety
Nanoparticle
Oxidative stress

ABSTRACT

Objective(s): MgO nanoparticles (MgO NPs) could be effective on anxiety-related behaviors in animal models but their exact mechanism of action compared to their conventional form (C MgO) is unclear. This study investigated and compared the effect of MgO NPs and its conventional form on anxiety-related behavior in the sleep-deprived rats and biochemical parameters changes in the serum.

Methods: Male Wistar rats (180-220g) were divided into groups of control, sleep deprivation induction, and 72 h sleep deprivation+ different doses of MgO NPs or C MgO. Components were injected a day after sleep deprivation induction and 30 min after injection animals passed anxiety test. Multiple platforms method was used for sleep deprivation induction. Serum oxidant/ antioxidant parameters, magnesium, and brain-derived neurotrophic factor were assessed after anxiety test.

Results: Sleep deprivation decreased anxiety-related behavior without change in the serum biochemical parameters. The anxiolytic effect of sleep deprivation decreased in the MgO NPs 1 mg/kg group. C MgO showed limited antioxidant activity, while MgO NPs did not change oxidant /antioxidant parameters, just the serum magnesium increased in the MgO NPs 10 mg/kg group.

Conclusions: MgO NPs changed anxiety behavior without changing the oxidant/ antioxidant factors, while C MgO changed some antioxidant factors but did not affect anxiety behavior. Therefore, the place or extent of action of MgO NPs and C MgO on anxiety-related behaviors is different (central or peripheral), which needs more investigation.

How to cite this article
Kesmati M., Nabaee E., Shahriari A., Torabi M. Comparative effects of MgO nanoparticles and conventional MgO on anxiety and serum biochemical parameters in sleep deprivation model of rat. Nanomed Res J, 2024; 9(2): 164-171. DOI: 10.22034/nmrj.2024.02.005

INTRODUCTION

Todays, nanotechnology has been expanded and used nano scale components with size less than 100 nm in drug delivery, tissue engineering, and diagnosis such as imaging [1, 2]. Despite the widespread application of nanoparticles in medicine and even at the cellular level, many of their side effects on central nervous system (CNS) activity and especially behavioral responses are still unknown [1-3].

Nanoparticles can easily pass through the blood-brain barrier, reach the brain and produce different physiological effects [4]. Magnesium oxide nanoparticles (MgO NPs) have been used in biological research, and the efficacy of MgO NPs can be different compared to conventional MgO (C MgO) on the CNS and behavioral responses in animals [5-7].

MgO NPs could influence behavioral parameters including anxiety, learning and memory, pain perception, depression and motor activity and even change gene expression, biochemical factors and neurotransmitters level in

* Corresponding Author Email: *m.torabi@scu.ac.ir*

the CNS of laboratory animals [5-8]. MgO NPs can change oxidative stress markers in the body [9, 10]. It is while, magnesium ion plays several important roles in many biochemical reactions of the body and magnesium is essential for neural protection and its deficiency can increase oxidative stress and impact sleep quality [11-14].

Magnesium ions did not easily pass through the biological barriers, and systemic treatment of the magnesium causes little change in its ion level of the cerebrospinal fluid [15].

So that, comparing the effect of elements in nano with their conventional forms in physiological processes can increase our knowledge regarding the advantages and disadvantages of nanoparticles action. With this flaw, and to study the effects of magnesium ion on the CNS activity, we need compounds that can increase the loading of this ion in the brain.

On the other hand, sleep is a phase of a circadian cycle in which the level of consciousness decreases and is easily reversed [16]. Sleep is a physiological process for the maintenance of health quality and sleep deprivation can lead to anxiety, as well as, induce oxidative stress, and impair the antioxidative mechanisms of the body [17-19]. Also, some studies have reported positive results about the impact of magnesium on insomnia and sleep indices [15, 20, 21].

Previously, we have shown that sleep deprivation causes memory deficits and MgO NPs improved memory, and eliminated anxiety better than C MgO in animal models [6, 7, 22].

In this study, for the first time we have try to clarify whether sleep deprivation affect anxiety-related behaviors and whether MgO NPs, compared to their conventional form, can play a role in improving anxiety-related behaviors in the sleep deprivation (SD) model of rats and biochemical parameters changes in the serum of them.

MATERIALS AND METHODS

Animals and treatments

Male Wistar rats (180-220 g) were divided randomly into groups of: control (saline), 24, 48, and 72 h SD, 72 h SD+ MgO NPs (1 mg/kg), 72 h SD+ MgO NPs (5 mg/kg), 72 h SD+ MgO NPs (10 mg/kg), 72 h SD + C MgO (1mg/kg), 72 h SD + C MgO (5 mg/kg), and 72 h SD + C MgO (10 mg/kg). MgO NPs (US Nano Co, USA) (particle size

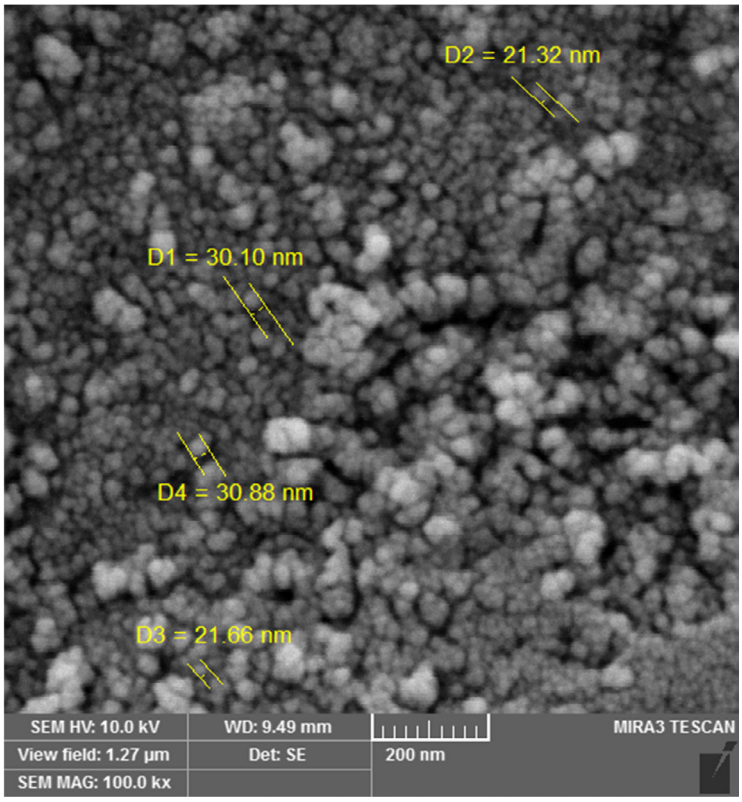

Fig. 1. The scanning electron microscopic image of MgO NPs. The size of particles is lower than 100 nm

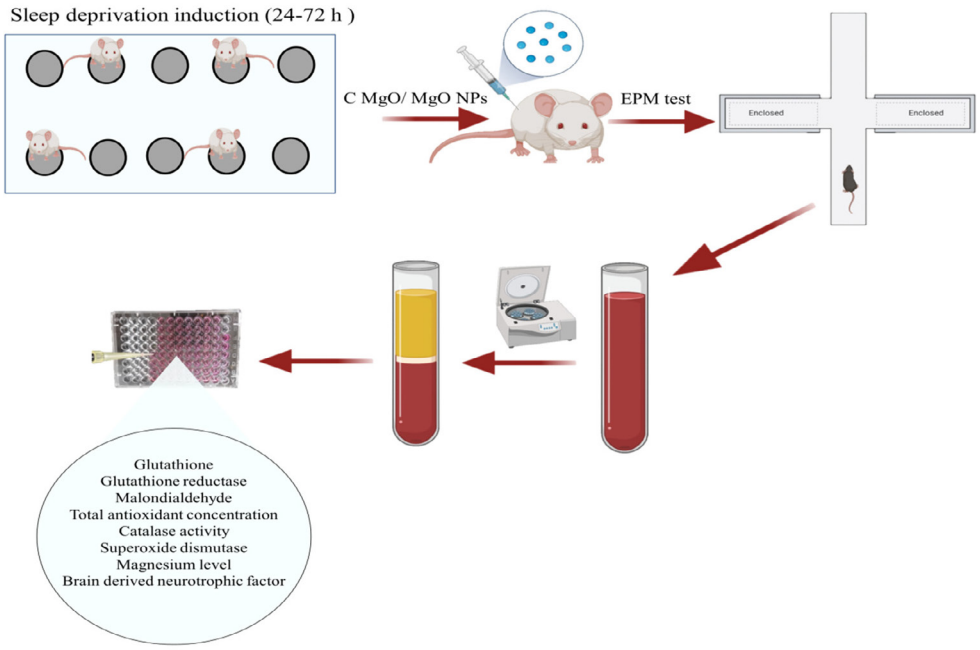

Fig. 2. Protocol of study. EPM= elevated plus maze; C MgO= conventional magnesium oxide; MgO NPs= magnesium oxide nanoparticles.

<50 nm) (Fig 1) and C MgO (Merck Co, Germany) (particle size >100nm) were dispersed in normal saline and injected intraperitoneally (1 ml/kg).

Drugs were injected into rats 24 hours after SD induction, and animals were subjected to the elevated maze (EPM) test, 30 minutes after injection. Number of animal in each group was six. All procedures were done under ethical code number of EE/96.24.3.88375/scu.ac.ir, approved by the Ethics Committee of the Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Figure 2 shows the study protocol.

Sleep deprivation

Sleep deprivation was induced by the multiple platform method, which uses a rapid eye movement technique to manipulate sleep deprivation. Each tank has ten columns, which designed in two rows and spaced 10 cm apart. Rats were placed in each tank for different hours, as previously described [23].

Elevated plus maze (EPM)

Anxiety parameters were measured by the EPM test. The EPM has two cross shaped open and closed arms without a roof, which were connected by a central square. Animals were placed in the central square facing the open arm for testing and were allowed to explore the maze. The anxiety-related parameters including the percentage of open arm time (%OAT) and the percentage of open arm entries (%OAE) were investigated during 5 min. The motor activity of each rat was calculated with the total number of animal entrance to the closed arms.

Serum biochemical parameters measurement

After completing the EPM test, all rats were killed using chloroform according to the instructions, and whole blood was immediately collected through cardiac punctuation, and then centrifuged to obtain the serum.

Brain-derived neurotrophic factor (BDNF) was measured using ELISA (Enzyme-Linked Immunosorbent Assay) Kit method (CK-E30666 produced by East Bio pharm) in the serum. Total antioxidant capacity (TAC), superoxide dismutase (SOD), Catalase activity, glutathione (GSH), and malondialdehyde (MDA) levels were measured according to the methods have been described in our previous study (7, 22). The serum magnesium level was measured using a magnesium kit (Pars Azmoun, Iran).

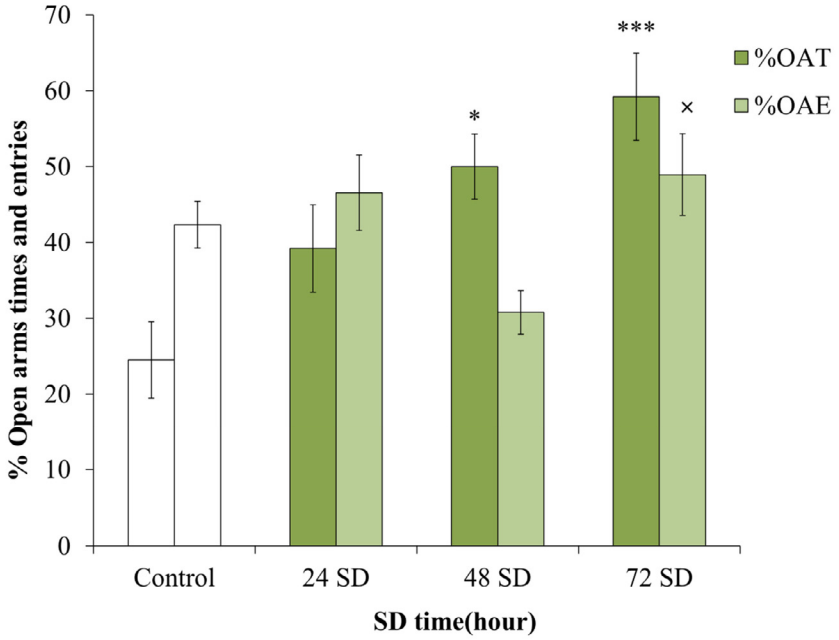

Fig. 3. Effects of sleep deprivation on anxiety-related behaviors. *p<0.05 and *** p<0.001 show the significant differences compared to control group at the same parameter. ×p<0.05 shows the significant difference compared to 48 h SD at the same parameter.

Statistical Analysis

Data were analyzed by the Instat 3 software. One-way analysis of variance (ANOVA) with Tukey Post-hoc was used for data analyses and a P-value less than 0.05 was considered statistically significant.

RESULTS

Effects of sleep deprivation on anxiety-related behaviors

Figure 3 shows that the %OAT significantly (p=0.05 and p=0.001, respectively) was increased in 48 and 72 h SD groups, while 24 h SD had no effect. Also, 72 h SD increased %OAE compared to the 48 h SD (p =0.0342).

Figure 4 shows that 48 h SD increased motor activity even thought it was not significant.

Effects of C MgO and MgO NPs on anxiety-related behaviors in sleep-deprived rats

Figure 5 (A and B) shows that in 72 h SD rats, %OAT (p=0.181) and %OAE (p= 0.296) were not affected by the C MgO, while % OAT was decreased by the MgO NPs 1 mg/kg (p=0.0018).

Figure 6 shows that in 72 SD rat, motor activity was not affected by all doses of C MgO (p=0.0867) and MgO NPs (p=0.0759).

Serum biochemical factors

Catalase activity (p= 0.3826), TAC (p= 0.0528), GSH level (p= 0.1766), SOD activity (p= 0.2641), MDA level (p= 0.0861), and BDNF level (p= 0.3592) were not affected by the sleep deprivation (Table 1).

Table 1 shows TAC (p = 0.5268), SOD activity (p= 0.1898), MDA level (p= 0.4083) and BDNF level (p= 0.4398) were not affected by C MgO. But, Catalase activity (p= 0.033) was significantly increased in C MgO 1 mg/kg in comparison 72 h SD group and GSH level (p =0.0482) were changed significantly between C MgO 1 and 10 mg/kg groups.

Catalase activity (p=0.5104), TAC (p= 0.2335), GSH level (p= 0.359), SOD activity (p=0.2498), MDA level (p=0.4083), and BDNF level (p= 0.9112) were not changed significantly by the MgO NPs injection in 72 h SD rats.

Sleep deprivation and C MgO had no effect on serum magnesium levels (p= 0.0537 and p= 0.3624, respectively), while magnesium level was increased by the MgO NPs 10 mg/kg (p= 0.0435) (Table 1).

DISCUSSION

In this study, sleep deprivation significantly decreased anxiety parameters; as well as 48 h sleep

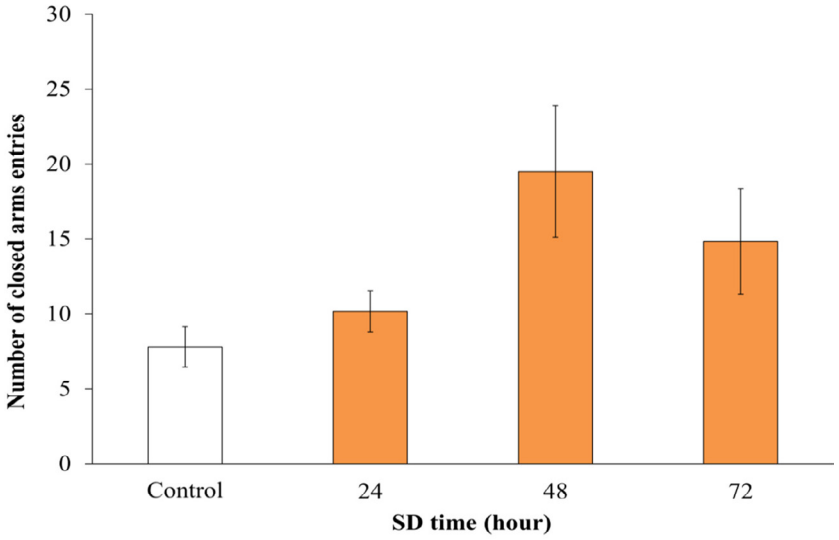

Figure 4. Effects of sleep deprivation on motor activity. No difference was observed between the groups.

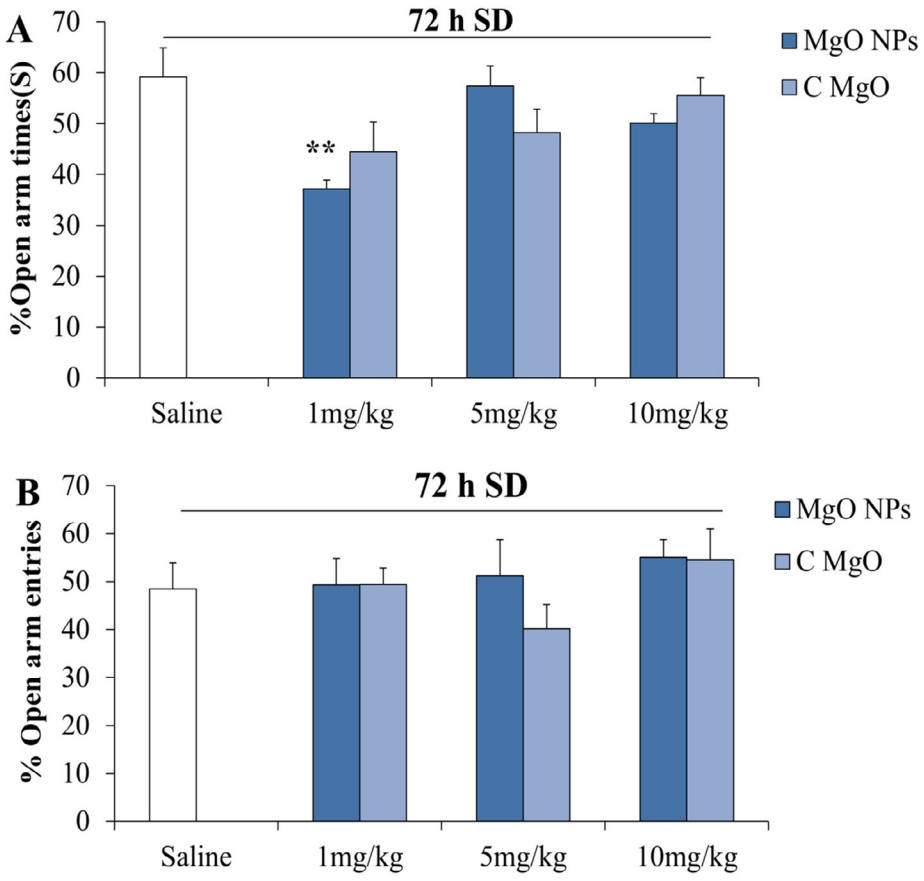

Fig. 5. Effects of MgO NPs and C MgO on anxiety-related behaviors in sleep-deprived rats. **p<0.01 is compared to the saline group.

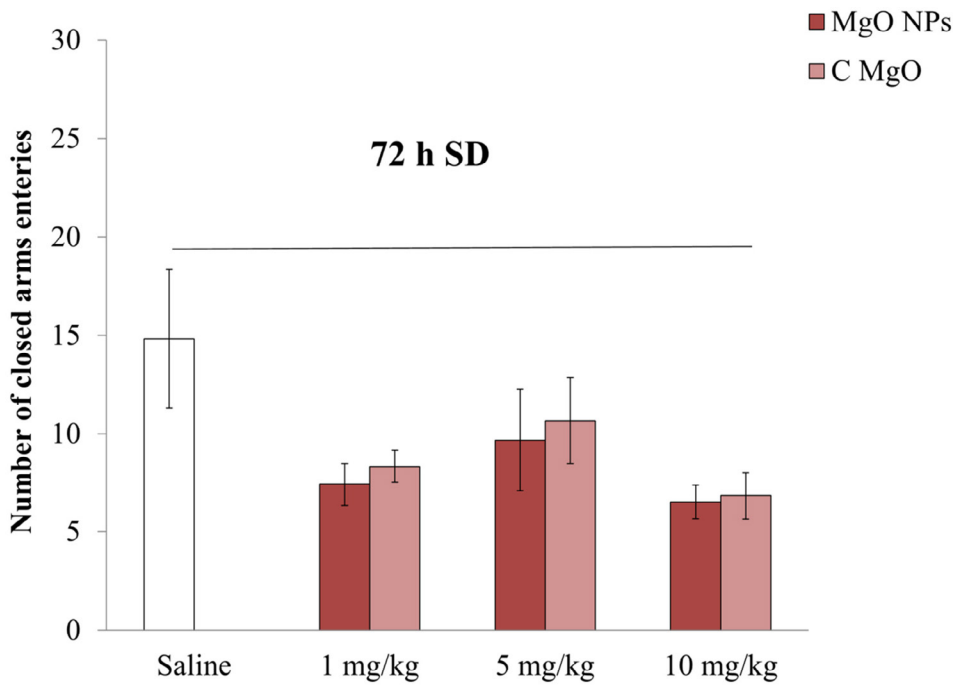

Fig. 6. Effects of MgO NPs and C MgO on motor activity in sleep-deprived rats. No significant differences were observed between motor activities of all groups.

Table 1. Serum biochemical factor in all groups

	Groups	Catalase (Iu/l)	TAC (µmol/l)	GSH (µmol/l)	SOD (Iu/l)	MDA (µmol/l)	BDNF (ng/mg pr)	magnesium(mg/dl)
	Control(saline)	1.16303 ±0.4272	759.2 ±67.615	71.08571±11.957	1.081272±0.01986	3.759717 ±0.1814	1.150714± 0.1065	2.1154± 0.02
	24 h SD	0.702307±0.4166	855.5± 54.843	76.51786±7.695	1.090106±0.01272	3.094529±0.1856	1.025776± 0.1079	2.1158± 0.01
	48 h SD	1.404411±0.3406	604 ±29.16	41.42857±5.977	1.035337±0.03085	3.223498 ±0.07462	0.840667 ±0.1724	2.0563± 0.02
	72 h SD	0.5974454± 0.1266	772± 73.624	62.28571±16.526	1.1053 ±0.03141	4.19788 ±0.5670	1.141192±0.1403	2.1698± 0.03
72 h SD+	C MgO 1 mg/kg	1.9375193 ±0.2256**	808.25 ±86.348×	101.2142 ±19.166	1.141343552±0.01619	3.413428 ±0.07133	2.22±0.02	2.22±0.02
72 h SD+	C MgO 5 mg/kg	0.979631 ±0.2624	589.5333 ±15.147	55.96429 ±3.858	1.077032552 ±0.01754	3.756184 ±0.1574	2.19±0.04	2.19±0.04
72 h SD+	C MgO 10 mg/kg	1.209546 ±0.3977	538.4 ±58.626	44.84286 ±8.779	1.115194552 ±0.004688	3.696113 ± 0.2449	2.25±0.03	2.25±0.03
72 h SD+	MgO NPs 1 mg/kg	1.37555 ±0.2021	700.2 ±23.161	77.51786 ±8.974	1.091873 ±0.01193	3.630177 ±0.1795	2.17±0.02	2.17±0.02
72 h SD+	MgO NPs 5 mg/kg	0.921865 ±0.2576	583 ±82.621	51.07143 ±3.134	1.132155552 ±0.02572	3.992933 ±0.3287	1.14349 ±0.1088	2.18±0.04
72 h SD+	MgO NPs 10 mg/kg	1.225949 ±0.6498	658.6 ±55.08	63.9 ±6.701	1.164664552 ±0.03076	3.886926 ±0.3638	1.129371± 0.129	2.28±0.02*

*p<0.05 and ** p<0.01 are the significant difference compared to 72 h SD group. *p<0.05 is a significant difference compared to C MgO 10 mg/kg.

deprivation increased closed arms entry which was not significant.

Serum oxidative stress markers were not changed by the sleep deprivation, just 72 h SD briefly decreased catalase activity and increased MDA level in the serum of rats.

Although one of the most critical outcomes of sleep deprivation is an increase in anxiety levels, there is no general agreement on the correlation between the different types of sleep deprivation and anxiety level [24]. There are experimental evidences denoting that anxiety could be negatively influenced by the sleep loss [25-27]. Garg and colleagues showed that 72 h SD significantly

increased anxiety levels in the EPM test [19]. In our previous study, 72 h SD could impair memory in rats, so that probably losing memory could be adequate in anxiety decrement [23].

There is evidence that in the multiple platforms method because of methodological issues, animals can move freely on platforms and eliminate the social isolation stress; in contrast, a single platform generates immobilization and oxidative stress due to movement restriction and social isolation [16]. Atrooz and Salim have reported that sleep deprivation had no oxidative damage in rats [28]. By contrast, Garg and colleagues showed that 72 h SD increased lipid peroxidation, decreased glutathione levels and catalase activity in mice [19].

On the other hand, C MgO and MgO NPs did not affect anxiety indices and motor activity in 72 h SD rats, and only the lowest dose of MgO NPs significantly decreased the anxiety index (Fig 5& 6). As well as, C MgO showed limited antioxidant activity with increasing catalase and total antioxidant level, while significant changes were not observed following MgO NPs administration in serum oxidative stress markers (Table 1).

There is a doubt on nanoparticles usage, in one side magnesium is necessary for neuroprotective and on the other side nanoparticles can change oxidative stress parameters in animals [6, 7, 9,10,13,]. Our results showed that although MgO NPs 1 mg/kg could reverse the anxiolytic effect of 72 h SD in rats, but did not cause significant oxidative stress changes. Notably, catalase activity increased in the serum of MgO NPs treated groups, specifically in the MgO NPs 1mg/kg group, but it was not significant.

In this study, serum magnesium levels were not changed by the sleep deprivation. Also MgO NPs and C MgO increased serum magnesium levels and this increment was only significant in MgO NPs 10 mg/kg treated group.

Our results showed that the serum BDNF level had not any significant changes in all groups; just it was briefly increased in MgO NPs 1 mg/kg group. Contrary to our results, Torabi-Nami and colleagues revealed that total and chronic partial sleep restriction could significantly decrease serum BDNF levels [27]. Some scholars showed a negative correlation between BDNF concentration and anxiety [29, 30]. Nevertheless, our results showed anxiety was decreased by the 72 h SD, without changes in the serum BDNF concentration. In line with our study, Alzoubi and colleagues showed that SD cannot affect the BDNF level in the hippocampus [31].

CONCLUSION

In conclusion, these results suggested that decrement of anxiety level following sleep deprivation induction in rats is not related to the serum oxidant/ antioxidant markers, magnesium, and BDNF levels. Also, administration of MgO NPs could reverse anxiolytic effects of sleep deprivation, whereas did not change serum biochemical factors. In contrast, the C MgO administration could change some antioxidant factors but did not affect anxiety related parameters. It seems that, the place or extent of MgO NPs and C MgO action on anxiety-related behaviors is different (central or peripheral), which needs more research.

ACKNOWLEDGEMENTS

We would like to acknowledge the Shahid Chamran University of Ahvaz for their financial support (grant number= 98/3/05/14909).

CONFLICT OF INTERESTS

NO

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