Effect of zinc oxide nanoparticles along with vitamin C on motor activity and anxiety in adult male rat

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Abstract

Introduction: Regarding the many applications of nanomaterials in various industries and the existence of many hypotheses on the harmful effects of nanoparticles on living things, the research in this field is of great significance. Thus, the present study aimed to investigate the effect of zinc and vitamin C nanoparticles as antioxidants and administration both on locomotor activity and anxiety.

Materials and methods: In this experimental study, rats were divided into seven groups including one saline group and six treatment groups. Zinc oxide nanoparticles (ZnO) were injected intraperitoneally in six groups at three concentrations of 1.25, 2.5, 5 mg/kg and vitamin C at three concentrations of 30, 60 and 120 mg/kg. At the end of the prescription period, the number of lines crossed in the open field test for motor activity and the number of stools for anxiety in each group were evaluated over a 5-min period.

Results: Prescription of different doses of ZnO and vitamin C did not indicate any significant change in motor activity compared to the saline group. The injection of 30 and 120 mg/kg of vitamin C decreased the number of defecation (anxiety) compared to the saline group ($P < 0.001$and $P < 0.01$, respectively) and also significant decrease was observed at 5 and 1.25 mg/kg doses of ZnO compared to the saline group ($P < 0.001$).

Conclusion: Based on these observations, vitamin C and ZnO reduced anxiety but had no effect on the motor activity of animals.

Keywords: Zinc Oxide Nanoparticles, Vitamin C, Motor Activity, Anxiety, Rat

Introduction

Motion and motor behavior is one of the most complex physiological phenomena playing a critical role in the survival and life of animals. Motor behavior is significant in creating environmental competitiveness, flexibility in responding to social change and non-social situations, aggressive behaviors, conflict and escapism, and especially in the reproduction (1). Depression and anxiety are among the common non-motor symptoms in patients (2). Motor activity and anxiety are regulated in various neurological centers include, hippocampus (3), hypothalamus (4). Anxiety is considered as a natural response to stress giving the person the ability to cope with difficult situations. When the severity and duration of anxiety symptoms prolong, it may become one of the types of pathological anxiety which may require treatment. Such conditions are typically related to stressful life experiences, especially when the stresses are chronic and traumatic (5). Studies indicated that the disruption of the balance of the neurotransmitter system, changes in

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signal transmission path, and the deformation of brain neuronal circuits contribute in this pathology (6). Anxiety disorders are associated with the hypothalamic-pituitary-adrenal axis (7). GABAergic and serotonergic systems are significantly involved in the regulation of the anxiety system (8). The activation of the GABA receptor improves anxiety disorders (9). Some studies indicated that zinc deficiency in the body can be the cause of anxiety behaviors (10). However, feeding with some organic and inorganic zinc supplements such as zinc sulfate, ZnSO4, conventional ZnO, and Zn methionine has partially improved anxiety in animal models (11). Zinc oxide is an inorganic compound being widely used in various applications, especially in pharmaceutical applications, as well as technical and cosmetic products. (12). Zinc oxide stimulates the growth and activity of various enzymes in the body and also affects many receptors such as serotonin, GABAergic receptor, and NMDA and calcium voltage-dependent channel (13). The mentioned receptors have essential roles in the modulation of anxiety. ZnO is one of the most important compounds for drug use due to its unique properties. For example, it is used as a carrier of various drugs, agents stimulating neuronal growth, antimicrobial agents and its analgesic effect was recently shown (14). Vitamin C, or ascorbic acid, is found in the water-soluble vitamins group. Ascorbic acid accumulates in the mammalian brain more than any other tissue. Ascorbic acid concentrations are more than 250 µg/g of tissue in a large number of anterior brain structures such as the hippocampus, nucleus accumbens, corpus striatum, hypothalamus, and septum (14). Vitamin C is required to have a good mental health. The lack or deficiency of this vitamin can cause depression, dizziness, heart disease, anxiety and fatigue. Anxiety and excitement increase ascorbic acid loss. Depression and anxiety may be exacerbated by inadequate absorption of ascorbic acid. Vitamin C is an anti-stress vitamin and may antagonize with adrenaline (15). Studies indicated that vitamin C has a synergistic effect on the anxiolytic and antidepressant effects in their low doses (16). In this regard, this study aimed to investigate the effect of ZnO and vitamin C on motor activity and anxiety in adult male rats.

Materials and methods

In this study, the adult male rats of Wistar race (200 to 250 g) which were prepared from Laboratory Animal Reproduction Center of Ahvaz Jundishapur University of Medical Sciences were used. The rats were kept one week prior to the start of any test to comply with the storage environment (animal storage room) at the standard conditions of 22±2 °C and 12-hour lighting cycle (Lighting hour of 7-19) and they had free access to water and food. The animals were divided into experimental and saline groups. Collectively, seventy adult male rats were randomly divided into ten groups with seven rats in each group (n= 7).
ZnO was injected intraperitoneally into the experimental groups (1.25, 2.5, 5 mg/kg for each of the tested groups) and also vitamin C was injected intraperitoneally into the groups (30, 60 and 120 mg/kg). For saline group, only 0.9% saline (10 ml/kg) was injected (17). Open field test was used to investigate motor activity in animals. The test equipment included a rectangular plate made of wood which its bottom was divided into 16 squares with lines. At first, each rat was placed at the center of the plate and its behavioral activity was monitored for five minutes. At the end of the test, each rat was removed from the open field and the test chamber was completely cleaned and dried with a damp cloth (18).

Statistical analysis

SPSS software and LSD test were used for statistical analysis. One-way ANOVA was used to compare the effects. Data were stated as mean ± SD and P value less than 0.05 was considered significant.

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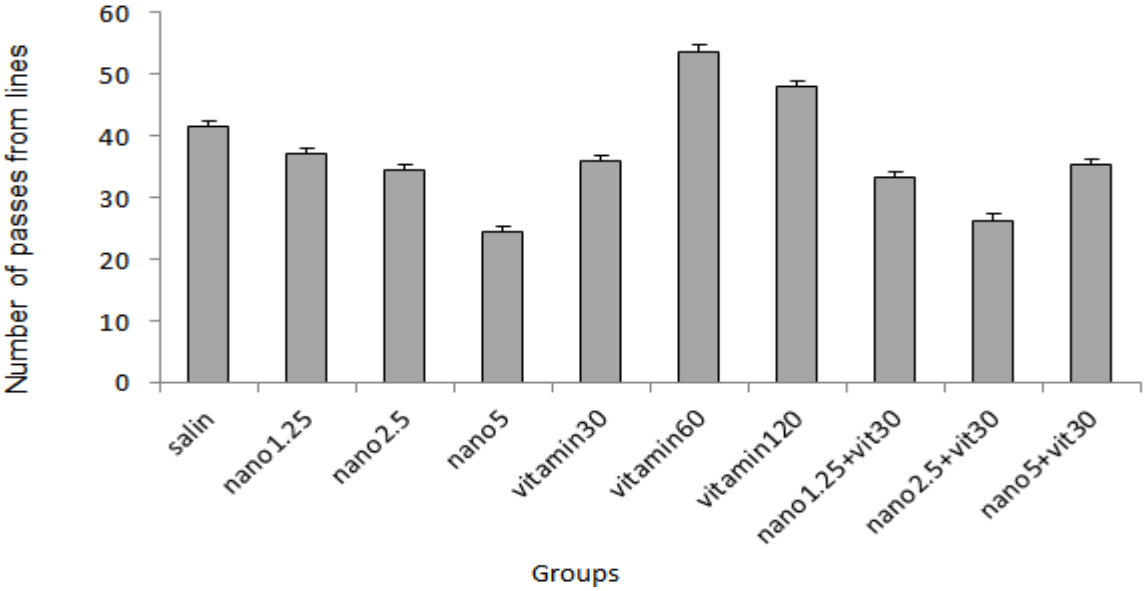


Figure 1. Comparison of the number of passes from lines in open field test between rats receiving doses of 1.25, 2.5, and 5 mg/kg of ZnO and 30, 60 and 120 mg/kg of vitamin C and different doses of ZnO NPs and vitamin30 compared to the saline (mean ± SEM; One-way ANOVA and Tukey's test (n=7)).

Results

As shown in Figure 1, the rats receiving doses of 1.25, 2.5, and 5 mg/kg of ZnO compared to the saline group failed at showing a significant difference in the number of passes from lines in open field test. The graph showed that the rats receiving 30, 60 and 120 mg/kg of vitamin C compared to the saline group failed at showing a significant difference in the number of passes from lines in open field test. On the other hand, the prescription of various doses of ZnO NPs and vitamin C did not indicate any significant difference in the number of passes from lines in open field test.

Effect of intraperitoneal injection of ZnO on anxiety behaviors

Figure 2 shows the ZnO injections at doses of 1.25, 2.5, 5 mg/kg for 14 days on the number of defecation in the open field test. One-way ANOVA indicated a significant decrease in the number of defecation at doses of 5 and 1.25 mg/kg compared to the saline group ($P < 0.001$).

Effect of intraperitoneal injection of vitamin C on anxiety behaviors

Vitamin C was injected at doses of 30, 60, 120 mg/kg for 14 days on the number of defecation in the open field test. The results revealed that the intraperitoneal injection of 30 and120 mg/kg dose of vitamin C decreased the number of defecation (anxiety) compared to the saline group with ($P < 0.001$), ($P < 0.01$).

Effect of intraperitoneal injection of ZnO and vitamin C (30 mg/kg) on anxiety behaviors

As indicated in Figure 2, the combined injection of 30 mg/kg ascorbic acid and 1.25 mg/kg ZnO significantly decreased the number of defecation in the open field test compared to the saline group ($P < 0.05$), while it failed at showing a significant increase at the dose of 30 mg/kg with ZnO of 2.5 and 5 mg/kg. There was no significant difference between the groups receiving all three doses of nanoparticles alone and the three doses of nanoparticles in vitamin C. composition.

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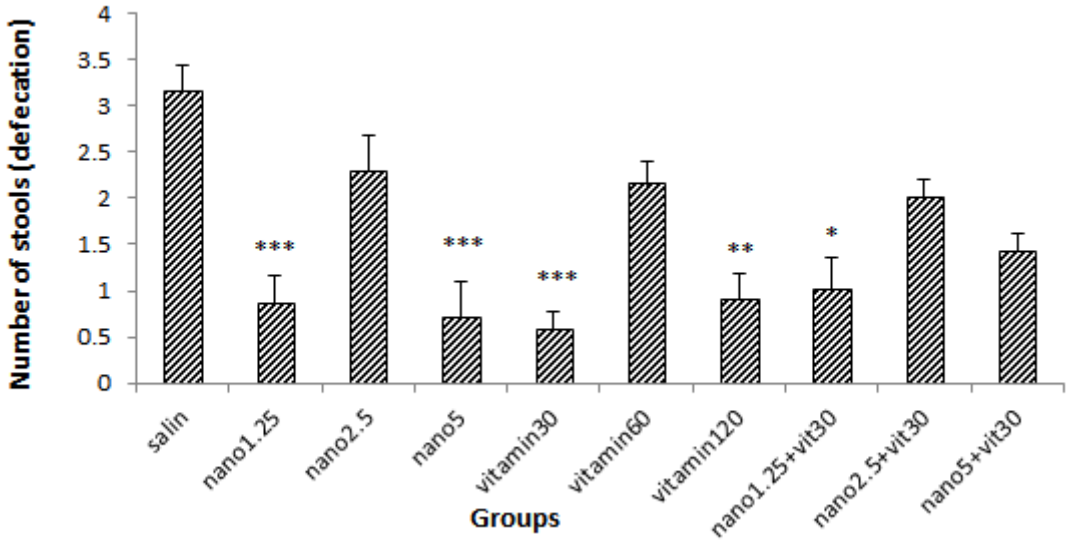


Figure 2. Comparison of the number of defecation in open field test between groups receiving doses of 1.25, 2.5, and 5 mg/kg of ZnO and 30, 60 and 120 mg/kg of vitamin C and different doses of ZnO NPs and vitamin30 compared to the saline , (mean ± SEM; One-way ANOVA and Tukey's test (n=7)). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Discussion

The results of this study revealed that ZnO significantly reduced anxiety and had no effect on motor activity of animals. Previous studies indicated that the cute application of zinc oxide and ZnO reduced anxiety and the anxiolytic effect of ZnO was obtained in less value than that of conventional ZnO, suggesting that ZnO was more efficient than the conventional type (19). Previous studies showed that 5 and 10 mg of zinc oxide and ZnO and even 25 mg of ZnO had no effect on motor activity (20). Previous studies indicated that ZnO had a weakening effect on motor coordination (12). Studies on the effect of different levels of zinc chloride on motor balance and behavior in young male rats showed that the rats which had consumed 50 or 30 mg/kg/day of zinc chloride for two weeks had more balance than the saline group and they spent more time on the rotarod device showing a significant difference with each other. On the other hand, the higher levels of zinc chloride initially affected motor activity, balance, and degenerative activities, but they continued to cause significant reductions. Zinc can have a dampening effect on motor activity and balance under conditions such as high amounts, and, due to the change in the behavior of nanoparticles, it can be suggested that one of the probabilities of ZnO destructive effect on the balance may be related to the size of this compound. Some studies indicated that nano-size compounds can reach the brain and may be associated with neurodegenerative diseases (21). Studies showed that the zinc ion has an inhibitory effect on glutamate signaling. In the presynaptic space, zinc and glutamate ions were released together (21, 22). Glutamate is one of the significant excitatory neurotransmitters in anxious behaviors and zinc ion with NMDA receptor inhibition as one of the major glutamate-target receptors attenuates glutamate function on anxiety. On the other hand, zinc ion lets the decrease of glutamate presynaptic exit and attenuates its signaling, thereby prevents anxiety by increasing GABAergic exit as inhibitory neurotransmitter, (22). On the other hand, some research indicated that a part of the anxiolytic effects of ZnO was related to the

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opioid system (22, 23). Based on the obtained data, the results showed that ascorbic acid at 30, 60 and 120 mg/kg doses reduced anxiety but did not affect motor activity at these doses. Ascorbic acid was introduced as a neuromodulator in the central nervous system (23) in addition to its cofactor and antioxidant role (23). Such results were consistent with other studies of ascorbic acid or studies that showed this vitamin has reduced anxiety. For example, Gautam et al. reported that the use of antioxidants such as vitamin E and C in patients with anxiety disorders led to an increase in antioxidant levels empirically, and the symptom of anxiety and depression were significantly reduced indicating the effect of vitamin C and E in reducing anxiety (24). Jesus et al. reported that the oral consumption of vitamin C supplementation in 42 high school students reduced anxiety levels and stated that a vitamin C-rich diet may be an effective adjunct to medical and psychological treatment of anxiety and improved academic performance (25). Oxidative stresses, which increased extracellular glutamate, increased the release of ascorbic acid from the cells until during the process of release, ascorbic acid and glutamate exchange, and its extracellular concentration regulate (26). The effect of vitamin C injection on anxiety indices showed that vitamin C alone had no effect on anxiety indices. (27). Based on such observations, vitamin C and ZnO reduced anxiety but had no effect on motor activity of animals.

Conclusion

Based on the open-field test performed in these groups, it can be concluded that this administration failed at affecting the motor activity of rats receiving different doses of zinc oxide even in the presence of vitamin C. Some doses of nanoparticles alone as well as vitamin C reduced the anxiety in the open-field test while their simultaneous administration had no effect on the number of excretions**.**

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References

1. Marczinski C, Perrot-Sinal TS, Kavaliers M, Ossenkopp KP. Sex differences in spontaneous locomotor activity and rotational behavior in meadow voles. Physiol Behav. 1998;65(2):387-91. doi: 10.1016/s0031-9384(98)00111-5.
2. Mollazadeh E, Khalafbeigi M, Zaree M, Taghizadeh G. Comparing the Single-item Visual Analog Scale with Multi-item Hospital Anxiety and Depression Scale, and Patient Health Questionnaire-9 to Diagnose Depression in Patients with Idiopathic Parkinson's Disease. Middle East J Rehabil Health Stud. 2017;4(4):e57484. doi: 10.5812/mejrh.57484.
3. Kobayashi K, Ikeda Y, Suzuki H. Behavioral destabilization induced by the selective serotonin reuptake inhibitor fluoxetine. Mol Brain. 2011;4:12. doi: 10.1186/1756-6606-4-12.
4. McNaughton N, Corr PJ. A two-dimensional neuropsychology of defense: fear/anxiety and defensive distance. Neurosci Biobehav Rev. 2004;28(3):285-305.
5. Alizadeh M, Hoseini M, Shojaeizadeh D, Rahimi A, Arshinchi M, Rohani H. [Assessing Anxiety, Depression and Psychological Wellbeing Status of

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Urban Elderly Under Represent of Tehran Metropolitan City]. Salmand Iran J Ageing. 2012;7(3):66-73.(Article in Persian)
6. Hajmohammadi F, Neshat Doost HT. [Comparison of spirituality in women with depression, anxiety and normal people]. JSRP. 2017;18(3):1-11. (Article in Persian)
7. Banaeipour Z, Rostami S, Zarea K, Cheraqian B. The prevalence of anxiety and its related factors among school-age children in South West of Iran. Int J Pediatr. 2016;4:2019-25. doi: 10.22038/IJP.2016.6970.
8. Olivier JDA, Christiaan H. Vinkers, Olivier aB. The role of the serotonergic and GABA system in translational approaches in drug discovery for anxiety disorders. Front Pharmacol. 2013; 4(74). doi:10.3389/fphar.2013.00074.
9. Singewald N, Schmuckermair C, Whittle N, Holmes A, Ressler KJ. Pharmacology of cognitive enhancers for exposure-based therapy of fear, anxiety and trauma-related disorders. Pharmacol Therapeut. 2015; 149:150-90. doi:10.1016/j.pharmthera.2014.12.004.
10. Tassabehji NM, Corniola RS, Alshingiti A, Levenson CW. Zinc deficiency induces depression-like symptoms in adult rats. Physiol Behav. 2008;95(3):365-9. doi: 10.1016/j.physbeh.2008.06.017.
11. Saeid S, Reza V, Moghimi A, Abbasali N, Hadi E. Evaluation the Anxiolytic Effects of Zinc Supplemented Diet in the Elevated Plus-Maze Test. Res J Biol Sci.. 2008. 9(3): 964-7. doi: rjbsci.2008.964.967.
12. Rafieirad M, Valipour Chahardah Charic S. Effect of Zinc Oxide Nano-Particles on Motor Coordination in the Attendance of Vitamin C in Rats. Report Health Care. 2017;3(2):1-6.
13. Takeda A, Tamano H. Insight into zinc signaling from dietary zinc deficiency. Brain Res Rev. 2009; 62(1):33-44. doi:10.1016/j.brainresrev.2009.09.003.
14. Ansari SA, Husain Q, Qayyum S, Azam A. Designing and surface modification of zinc oxide nanoparticles for biomedical applications. Food Chem Toxicol. 2011; 49(9):2107-15. doi:10.1016/j.fct.2011.05.025.
15. Umadevi P, Sevanan M, Suganthi JS, Subakanmani S. Evaluation of antidepressant like activity of Cucurbita pepo seed extracts in rats. Int J Curr Pharm Res. 2011;3:108-13.
16. Khanna Ranjana S, Reena N, Deepti P, Shruti K, Hari DK. Markers of oxidative stress in generalized anxiety psychiatric disorder: therapeutic implications. J Stress Physiol Biochem. 2012;8(2):32-8.
17. Rafieirad M, Valipour-Chahardah-Charic S. [Evaluation of the simultaneous effect of zinc oxide nanoparticles and vitamin C on oxidative stress in rat cerebellum]. Feyz. 2018;22(3):274-82. (Article in Persian)
18. Rafieirad M. Pomegranate seed extract reduces ischemia induced anxiety in male rats. J Herbmed Pharmacol. 2017;6:85-9.
19. Torabi M, Kesmati M, eshagh harooni H, Najafzadeh H. Different efficacy of nanoparticle and conventional ZnO in an animal model of anxiety. J Neurophysiol. 2013;45.
20. Eizadi-Mood N, Pourabdian S, Fallah M. Effects of chronic Zinc Fume exposure on memory and cognition. Iran J Toxicol. 2010;3(3):317-23.
21. Black MM. Zinc deficiency and child development. Am J Clin Nutr. 1998;68(2 Suppl):464S-9S. doi: 10.1093/ajcn/68.2.464S.
22. Takeda A, Minami A, Seki Y, Oku N. Differential effects of zinc on glutamatergic and GABAergic neurotransmitter systems in the hippocampus. J Neurosci Res. 2004; 75(2):225-9. doi:10.1002/jnr.10846.
23. Torabi M, Kesmati M, Eshagh Harooni H, Varzi HN. Effect of Intra CA1 and intraperitoneal administration of Opioid

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receptor modulating agents on the anxiolytic properties of nano and conventional ZnO in male rats. Cell J. 2014;16(2):163-70.
24. Gautam M, Agrawal M, Gautam M, Sharma P, Gautam AS, Gautam S. Role of antioxidants in generalised anxiety disorder and depression. Ind J Psychiatr. 2012;54(3):244-7. doi: 10.4103/0019-5545.102424.
25. Jesus Lima de Oliveira I, Souza V, Motta V, Leme Da-Silva S. Effects of oral vitamin C supplementation on anxiety in students: a double-blind, randomized, placebo-salineled trial. PJBS. 2015;18:11-8.
26. Castro M, Caprile T, Astuya A, Giovanetti C, Reinicke K, Vera JC, et al. High-affinity sodium-vitamin C co-transporters (SVCT) expression in embryonic mouse neurons. J Neurochem. 2001; 78:815-23. doi: 10.1046/j.1471-4159.2001.00461.x
27. Alboghobeysh S, Khajehpour L, Kesmati M. [Involvement of opioid receptors and ascorbic acid in the improvement of anxiety-induced nicotine in adult male mice]. J Arak Univ Med Sci. 2018;21(3):5-13. (Article in Persian)

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