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# Nanoparticles of Zinc Oxide Reduces Acute Somatic Pain in Adult Female Wistar Rats

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***Abstract***

***Background*:** With appearance of nano particles as an important component in modern medicine, and considering to new properties of these components, study of their effects on human health is essential. Since zinc components influences mechanisms of nociception, the aim of this study was to evaluate the effect of nano zinc oxide as a new source of zinc and important components in pharmaceutical and hygienic cosmetic production on nociception in adult female rats.

***Materials and Methods*:** Female rats were divided into groups: control (receiving saline 0.9%) and receiving nano ZnO (0.5, 1, and 5 mg/kg). Hot plate and tail flick tests as models of somatic acute pain were used for evaluation of the pain. The mean of latency time in paw licking and tail withdrawal respectively recorded as nociception indexes in each test for every animal. The animal numbers in each group was seven.

***Results*:** In tail flick test, nano ZnO (0.5, 1 mg/kg) and in the hot plate test in dose of 0.5 mg/kg, induces significant analgesia ($p<0.05$) and with increasing of dose reduced its analgesic effect.

***Conclusion*:** It seems nano ZnO inhibit the nociception mechanisms and these analgesic properties are more efficient in the low doses. Probably by increasing dose of nano particles aggregation phenomenon prevent of anti-nociception effects of nano ZnO.



## Introduction

All the scientists have accepted this fact that nanotechnology is a new era of incorporation of various sciences like engineering, biology, chemistry, medicine and physics [1]. Some of nanoparticles are considered as an emerging compound in the progress of medicine and pharmacy. These compounds due to having high potential are increasing applied for different reasons such as specific treatment processes in medical and pharmaceutical studies and have made a new branch of nanotechnology which is known as nano medicine [2, 3]. Nowadays, the application of nanoparticles in biotechnology and modern medical science, considering their unique properties, could reveal new ways in treatment of many disorders, particularly in the central nervous system (CNS) [4, 5]. Nanoparticle of ZnO as a nano oxide-element because of its chemical and physical properties, could grab the attention of bio researches and pharmacists. This nanoparticle is widely using in cosmetics and health products as well as medical equipment and drug delivery [6, 7].

According to some biological studies onan ZnO can affect the function of the cells and different tissues, however, less research has been done on its effects on the CNS [5]. Bulk zinc oxide with the formula ZnO is an inorganic compound that has known as a supplement that has many applications in various areas. Less the toxic effect of long-term usage of ZnO rather than other zinc containing compounds such as sulfate or bicarbonate, has caused it is chosen as an appropriate supplement by food production enterprises [8]. According to studies carried out on Zn ion and some of its compounds, Zn has been found to have analgesic effects, on the contrary, in the low level of it in the plasma causes visceral and somatic pain [9, 10]. Studies have shown that low concentration of zinc in humans is associated with premenstrual tension and painful periods [11].

Based on researches on pain threshold in mice, there is no significant difference between pain thresholds in male mice with different phases of female mice, but, in female mice, the threshold of pain in pro-estrus and di-estrus phases is significantly lower than estrous phase [12]. On the other hand, women account of most of the consumers of nano ZnO-containing compounds in health and cosmetic products, therefore, the aim of this study was to investigate the effect of ZnO nanoparticles as a new containing zinc compound on the somatic pain threshold in female rats.

## Materials and Methods

In this experimental research, adult female Wistar rats (weighting 190±10 g) were purchased from Animal Production Center in Jondishapour University of Ahvaz, Iran, and before study, they were kept in quiet space without stress conditions, 12 hours light/dark cycle and temperature 22±2ºC. All of the experiments were done in

light phase, between 9-12 am. Every animal was tested just once. It is worth mentioning that rats were randomly selected and probably they were in different sexual phases. All the ethics of working with laboratory animals were done in accordance with the principles of Ahvaz University of Medical Sciences.

Nano ZnO with the diameter less than 70 nm was purchased from (Lolitech Co; Germany). For preparing injectable suspension, nano ZnO once dispersed in saline 0.9%, by ultrasonic bath for 15 minutes and after that, it was dispersed by Shaker instrument before of each injection.

In the first test to measure thermal pain tail flick test was used. For this reason, 28 female rats were divided into 4 groups (N=7) in order: control (saline 0.9% receiver), and nano ZnO receiver groups (0.5, 1, and 5 mg/kg). All injections were performed in the volume of 1 ml/kg. Each animal was placed horizontally in the particular container while its tail was hanging out. While the first parts of the tail was adjusted on the light-sensitive sector with constant temperature (48ºC). Thirty minutes after treatment the tail flick latency for each animal, were recorded 2 times with 10 minutes interval and the average was calculated as, latency time. If the animal does not respond to painful stimuli after 45 seconds (cut off time) to prevent damage, the animal was removed from the device. For the second test the hot plate test was used. In this test all the 4 groups were tested same as the tail flick test. Hot plate system is composed of an aluminum plate, which its temperature by a temperature sensor and a digital temperature controller is to be determined. According to the procedure of test, we put the animal on the device plate which was hot until (52±2ºC), and then put the key on.

As soon as we observed the reaction of animal to pain (licking feet), this reaction was recorded as a latency time in response or painless time. In this test cut off time was considered 60 s. To study the structure of the particles before injecting in the suspensions with concentrations of 5 and 10 mg of dry powder in 5 ml saline, imaging device scanning electron microscope (S 4160, Japan) was used and the structure of particles in the suspension and dry powder was investigated. Data analyzed by SPSS-16 and one way ANOVA with post hock LSD. In all experiments $p<0.05$ used as significant level and bars show mean±SD.

## Results

In tail flick test nano ZnO in lower doses (0.5, 1 mg/kg) show significant anti-nociception effect in compared to control group, while didn't observed any effect in higher dose. Also we observed significant differences between the dose of 0.5 and 5 mg/kg (Fig. 1). In hot plate test nano ZnO in lowest dose (0.5 mg/kg) show significant anti-nociception effect in compared to control group while in doses of 1 and 5 mg/kg hasn't any effect on pain conception (Fig. 2). So nano ZnO in low doses exerted appropriate anti-nociception effect in adult female Wistar rats and by increasing dose this effect reduced. To investigation of this topic scanning electron microscopy was done from different dose of nano particle of ZnO (5, 10 mg) dispersed in 5 ml saline before injection. Images of A, B and C in figure 3 show that almost all particles in dry powder (A) and suspensions are global, also nanoparticles in suspension have tend to aggregate and make colony and by increasing concentration from 5 mg (B) to 10 mg (C) wrought colony has been larger too.

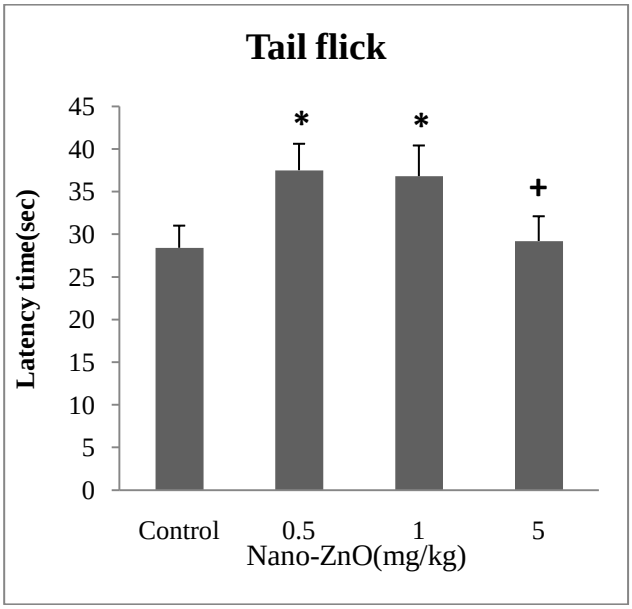


**Figure 1.** The effect of I.P. injection of nano-ZnO (0.5, 1, 5 mg/kg) on latency time in tail flick test in female rats. (*$p<0.05$) in compared to control group, (+$p<0.05$) in compared to nano-ZnO 0.5 mg/kg

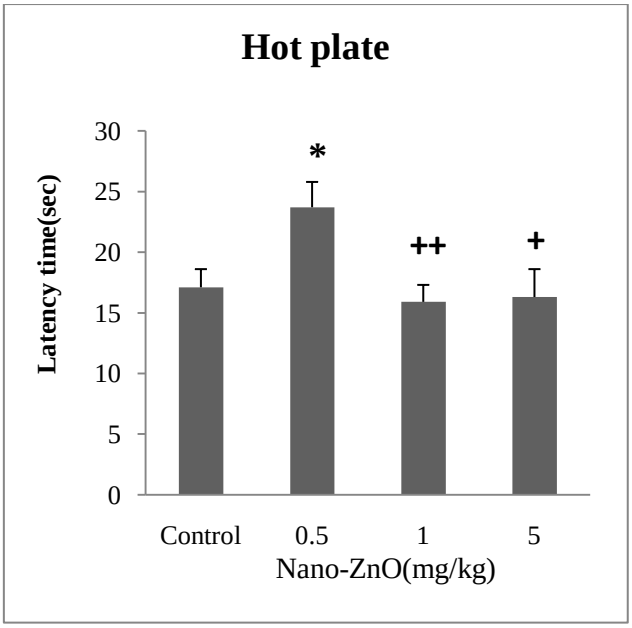


**Figure 2.** The effect of I.P. injection of nano-ZnO (0.5, 1, 5 mg/kg) on latency time in hot plate test in female rats. (*$p<0.05$) in compared to control group, (+$p<0.05$, ++$p<0.01$) in compared to nano-ZnO 0.5 mg/kg

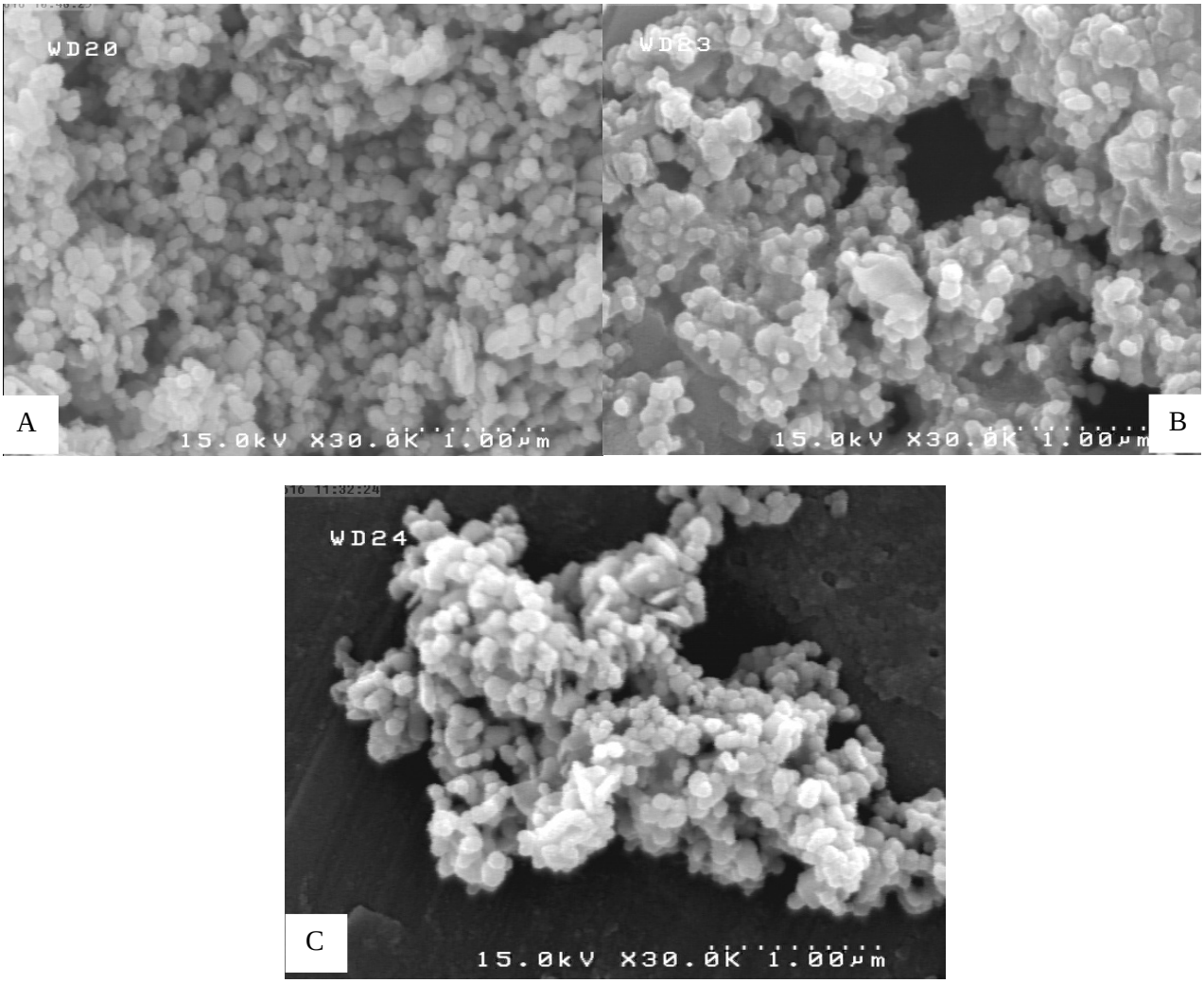


**Figure 3.** Scanning electron microscopy for determination of nanoparticle structure in dry powder (A), suspension with density of 5 mg dry powder/5 ml saline (B) and 10 mg dry powder/5 ml saline (C)

## Discussion

Results from this study, were shown that in tail flick test, nano ZnO in lower doses 0.5 and 1 mg/kg could reduce the pain in compared to control group. At the other hand in hot plate test, nano ZnO just in dose of 0.5 mg/kg exerted anti-nociception effect in female rats and in other doses hasn't any effect on nociception. According to our study, present finding is first result on investigation of the effect of nano ZnO on pain conception in female rat and hasn't accomplished any study in this field yet. Considering the importance of the acute pain suppressing, in this study hot plate and tail flick tests as models to make acute and somatic pain were used [13, 14]. These results have correlation with previous investigations that has done on the effect of zinc ion and some of its components on nociception. But in many studies male genus had been investigated. For example intra peritoneal injection of $ZnCl_2$ has led to suppressed neuropathic pain in male mice [15]. Intra-theca injection of $ZnCl_2$ has been led to reduce pain induced by acetic acid too. On the other hand intra-theca injection of zinc cheater has been led to increasing the pain of acetic acid in male mice [16]. Also has been shown that zinc deficiency by increasing the irritability of special ionic channels lead to increasing colonic pain as another visceral pain [17].

It has been demonstrated that Zinc ions there are in many amount in pre synaptic vesicles of glutamatergic neurons and co-release with glutamate as excitatory amino acid, controlled synaptic transmission that lead to reduce the release of glutamate by increasing the release of GABA as inhibitory neurotransmitter [18]. Also in vitro studies have shown that extra cellular zinc; affected on the collection of cell membrane signaling proteins that the main of them are NMDA receptors and have high sensibility for zinc and lead to inhibition of above receptors that are pre-nociception molecules [10, 19]. Glutamate role in transduction of pain signals from spinal cord to thalamus is important, glutamate by activation of NMDA receptor lead to increase of pain [20, 21]. It has been identified that NMDA receptors antagonists make anti-nociception effects by inhibit of this receptor. For example MK801 as NMDA receptor antagonist has anti-nociception effect at the under anesthesia doses and when

co-injected with morphine increase anti-nociception effects of morphine in male and female mice at hot plate test and week morphine tolerance in male mice [13]. Ketamine as another NMDA antagonist has analgesic effects [9]. Since it has identified that zinc like an antagonist inhibits the NMDA receptors, dependent on glutamate, and can make analgesic effects [10]. So it seems that in present study released zinc ion from nano ZnO has been down its analgesic effect in female rats by affecting on NMDA receptors and weak the work of it and/or reducing the release of glutamate by increasing the GABA level. But in answer to this question, why by increasing the dose of nano ZnO its analgesic effect reduced, it has been demonstrated that nanoparticles have high ability to make free radical species that these production can have undesirable effects on tissues and also it has been identified that in high density by increasing aggregation and accumulation of nanoparticles bioavailability of free ions from them reducing [22, 23]. In present study microscopic imaging of nanoparticles have shown that before injection particles in suspension have tendency to aggregation and rather than it by increasing the suspension density, more particles aggregate inside of each other (Fig. 3). Maybe occurrence of this phenomenon before injection in suspension has been reduced bioavailability of nanoparticle after injection in body. On the other side it has been demonstrated that receptors saturation by ion of zinc can inhibit increasing the response by increasing amount of zinc too [24]. So the reason of reduce nano ZnO analgesic effect by increasing concentration in present study can be every of membered reasons that identified main reason need to more investigations.

According to the results from this study it proposal that to induce analgesic effect of nano ZnO must attention to the usage dose. Also by attention to the role of zinc in analgesia mechanisms it seems that the mechanism of nano ZnO action is through the central mechanisms that this need to more investigation.

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### Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

### Conflict of Interest

The authors declare no conflict of interest.

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### *References*

1. Hatchett DW, Josowicz M. Composites of intrinsically conducting polymers as sensing nanomaterials. J Chem Rev 2008; 108(2): 746-69.
2. Dreher KL. Health and environmental impact of nanotechnology: Toxicology calassessment of manufactured nanoparticles. Toxicol Sci 2004; 77(1): 3-5.
3. Hardman RA. Toxicological review of quantum dots: Toxicity depends on physic-chemical and environmental factors. Environ Health Perspect 2005; 114(2): 165-72.
4. Hughes G, McLean NR. Zinc oxide tape: A useful dressing for the recalcitrant finger-tip and soft-tissue injury. Arch Emerg Med 1988; 5(4): 223-7.
5. Murthy SK. Nanoparticles in modern medicine: State of the art and future challenges. Int J Nanomedicine 2007; 2(2): 129-41.
6. Colvin V. The potential environmental impact of engineered nanomaterials. Nat Biotechnol 2003; 21(10): 11-66.
7. Wang ZL. Splendid one-dimensional nanostructures of zinc oxide: A new nanomaterial family for nanotechnology. ACS Nano 2008; 2(10): 1987-92.
8. Edwards HM 3rd, Baker DH. Bioavailability of zinc in several sources of zinc oxide, zinc sulfate, and zinc metal. J Anim Sci 1999; 77(10): 2730-35.
9. Martin D, Lodge D. Ketamine acts as a noncompetitive N-methyl-D-aspartate antagonist on frog spinal cord in vitro. Neuropharmacology 1985; 24(10): 999-1003.
10. Nozaki C, Vergnano AM, Filliol D, et al. Zinc alleviates pain through high-affinity binding to the NMDA receptor NR2A subunit. Nat Neurosci 2011; 14(8): 1017-22.
11. Sendur O, Tastaban E, Turan Y and Ulman C. The relationship between serum trace element levels and clinical parameters in patients with fibromyalgia. Rheumatol Int 2008; 28(11): 1117-21.
12. Kiasalari Z, Khalili MA. [Comparison of acute pain threshold between the male and female and the effect of Datura stramonium] Persian. Daneshvar 2008; 15(74): 59-66.
13. Bryant CD, Eitan S, Sinchak K, et al. NMDA receptor antagonism disrupts the development of morphine analgesic tolerance in male, but not female. Am J Physiol Regul Integr Comp Physiol 2006; 291(2): 315-26.
14. Le Bars D, Gozariu M, Cadden SW. Animal models of nociception. Pharmacol Rev 2001; 53(4): 597-652.
15. Liu T, Walker JS, Tracey DJ. Zinc alleviates thermal hyperalgesia due to partial nerve injury. Neuroreport 1999; 10(3): 1619-23.
16. Larson AA, Kitto KF. Manipulations of zinc in the spinal cord, by intrathecal injection of zinc chloride or disodium-calcium-EDTA, alter nociceptive activity in mice. J Pharmacol Exp Ther1997; 282(3): 1319-25.
17. Matsunami M, Kirishi S. Chelating luminal zinc mimics hydrogen sulfid-evoked colonic pain in mice: Possible involvement of T-type calcium channels. Neurosci 2011; 181: 257-64.
18. Takeda A, Minami A, Seki Y and Oku N. Differential effects of zinc on glutamatergic and GABAergic neurotransmitter systems in the hippocampus. J Neurosci Res 2004; 75(2): 225-29.
19. Mony L, Kew JN, Gunthorpe MJ and Paoletti P. Allosteric modulators of NR2B-containing NMDA receptors:

Molecular mechanisms and therapeutic potential. Br J Pharmacol 2009; 157(8): 1301-17.
20. Tao YX, Gu J, Robert L and Stephens JR. Role of spinal cord glutamate transporter during normal sensory transmission and pathological pain states. Mol Pain 2005; 1: 30.
21. Zhuo M. Glutamate receptors and persistent pain: Targeting forebrain NR2B subunits. Drug Discov Today 2002; 7(4): 259-67.
22. Sharma V, Shukla RK, Saxena N, et al. DNA damaging potential of zinc oxide nanoparticles in human epidermal cells. Toxicol Let 2009; 185(3): 211-18.
23. Kool PL, Ortiz MD, van Gestel CA. Chronic toxicity of ZnO nanoparticles, non-nano ZnO and ZnCl2 to Folsomia candida (Collembola) in relation to bioavailability in soil. Environ Pollut 2011; 159(10): 2713-9.
24. Teisseyre A, Mercik K, Mozrzymas JW. The modulatory effect of zinc ions on voltage-gated potassium currents in cultured rat hippocampal neurons is not related to Kv1.3 channels. J Physiol Pharmacol 2007; 58(4): 699-715.

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