RESEARCH PAPER

Zinc/Iron Oxide Nanocomposites: Green Synthesis, Characterization, and Evaluation of their Effects on Anxiety in Rats Exposed to Noise Stress

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ABSTRACT

In the modern lifestyle, environmental stresses have substantially influenced human life. Evidence indicates that noise exposure can lead to anxiety-like behaviors. In recent years, advances in nanotechnology have favorably impacted industrial productions. In the present study, we aimed to evaluate the effect of Zinc/Iron oxide nanocomposites on anxiety-like behavior. Using gelatin, Zinc/Iron oxide nanocomposites were synthesized by the sol-gel modified method. Four groups of rats were exposed to sound stress for 12 days. Three groups received 1.25, 2.5, and 5 mg/kg of Zinc/Iron oxide nanocomposites before the noise stress. Also, three control animals received the same doses of the nanocomposites. One group of the stressed (ST) and control (CO) rats received saline. The animals were introduced to the elevated plus maze (EPM) for the assessment of anxiety. Entry to closed arms, duration of stay in open arms were considered for data analysis. We observed a significant difference between the behavior of the CO and ST animals so that the latter group showed more anxiety compared to the former one. Concerning the effect of Zinc/Iron oxide nanocomposites, the data analysis confirmed the positive effect of the dose of 2.5 mg/kg where the stressed animals treated with this dose of the nanocomposites considerably improved their normal performance in the EPM.

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INTRODUCTION

Noise pollution is considered one of the products of the modern lifestyle that causes great damage to various systems of the body including the nervous system [1]. Noise pollution can cause stress leading to anxiety and depression. Noise stress also increases the immobility time, which indicates anxiety-like behavior [2]. To prevent the negative effects of noise stress on the auditory system, behavior, and mental health, ambient noise should not exceed 55 decibels during the day and 40 decibels during the night [3, 4].

Nowadays, interdisciplinary studies in nanotechnology have opened a new window of wonder in the world of science and knowledge, and have made the new principles of engineering and human understanding in the biological and medical sciences to undergo a novel transformation [5]. Extensive use of nanoparticles in various fields causes environmental pollution [6]. Due to their high permeability, nanoparticles easily cross the blood-brain barrier (BBB) and they can enter the human body in large quantities [7]. Therefore, these substances may cause negative

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effects on humans and other organisms than ordinary substances may do [8]. Destructive or beneficial effects of nanoparticles on cognitive function and psychological behavior have recently been the target of extensive studies [9].

Animal and human bodies respond to stress in two ways: a) rapid response, during which the autonomic nervous system (ANS) is activated leading to the secretion of the catecholamines epinephrine and norepinephrine in the adrenal gland, that results in typical symptoms of stress, and b) slow response, during which the hypothalamic-pituitary-adrenal (HPA) axis is activated, leading to increased secretion of corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) [10]. ACTH stimulates the adrenal cortex, giving rise to the secretion of glucocorticoids (GCs). The most important of GCs are cortisol in humans and corticosterone in rats (CORT) [11]. It is shown that exposing pregnant rats to noise stress for two to four hours/day increase serum corticosterone level [12]. Also, the serum level of corticosterone increases significantly in offspring exposed to maternal separation stress [13].

Oxidative stress, which is caused by an imbalance between the formation of free oxygen radicals and the inactivation of these species by the antioxidant defense system, can cause oxidative damage to various intracellular and extracellular molecules [14]. These detrimental effects generally appear after exposure to a relatively large amount of reactive oxygen species (ROS). Oxygen radicals are generated in the body as byproducts of oxidative metabolism [10]. To protect the oxidative cycle and stability, cells have developed defense mechanisms that strike the right balance between antioxidant and oxidant molecules [15]. Zinc ions participate in the mechanisms of the enzyme superoxide dismutase, thus playing an important role in oxidative balance and anxiety control [16].

The effects of zinc and magnesium supplements on postpartum depression were investigated in a clinical study [17]. The results showed that magnesium and zinc reduced postpartum anxiety and depression [17]. As a reducing anxiety-like behavior factor, zinc oxide nanoparticles are more effective in reducing anxiety behaviors than conventional zinc oxide. It is also shown that iron oxide nanoparticles exerts a pronounced anti-anxiety effect in small amounts (1 mg/kg), however, its anti-anxiety effect decreases in large quantities (5 mg/kg) [16].

Metal nanomaterials have various applications in the biological and medical sciences[18]. The effects of metal nanoparticles on variables such as stress and anxiety were investigated in this study.

Due to their magnetic properties, high biocompatibility, and high catalytic ability, zinc/iron oxide nanocomposites are widely used in industrial, agricultural, and medical fields such as medical imaging, biotherapy, and photocatalysis [19]. Zinc and iron food supplements are also effective in reducing anxiety [17].

By substituting natural materials and food waste as a gelling as well as a capping agent, we can have a cleaner environment by eliminating expensive chemical compounds [20]. Gelatin is a substance obtained from the hydrolysis of bovine bones and cartilages. Gelatin components consist of 84% protein, 2% ash, and 14% moisture. Gelatin is hydrolyzed collagen [21]. It is only soluble in hot water and is insoluble in cold water, and after dissolving in water at 40 to 50 °C, it absorbs about ten times its volume of water [21].

The main constituents of gelatin are the essential amino acids, including glycine, proline, hydroxyproline, glutamic acid, alanine, arginine, and aspartic acid [21].

The existing carboxyl functional group in these amino acids can turn into carboxylate ions through exchanging electrons with the existing amine group [21]. Existing carboxylate ions can surround the metal cations, form bonds, and enter condensation reactions with other carboxylate and amine groups to form a dense gel. Then, oxide nanoparticles can be prepared through drying and heating the wet gel [22]. The main aim of the present study was the administration of synthesized zinc/iron oxide nanocomposites to an animal model of anxiety to assess if the nanoparticle can reverse the adverse anxiety behavior.

MATERIALS AND METHODS

Materials

The produced materials of Sigma-Aldrich, such as Iron (III) Nitrate Nonahydrate $Fe(NO_3)_3.9H_2O$ and Zinc acetylacetonate hydrate ($Zn(C_5H_7O_2)_2.6H_2O$) or $Zn(acac)_2$ and materials of Merc Co, include methanol and chloroform, were applied in this study. Also, colorless, odorless, and essential oil-free animal (edible) gelatins were prepared from the traditional medicine market.

Synthesis of zinc/ iron oxide nanocomposites (ZIONC)

In the present study, a modified sol-gel method was employed for the synthesis of ZIONC. Next, $Zn(acac)_2$ and iron nitrate were dissolved in 50 mL of deionized water in a 1:2 stoichiometric ratio and stirred magnetically for preparing a homogeneous solution. In the next step, 2g of natural animal gelatin was added to deionized water, stirred, and heated at 80°C to provide a viscous and uniform gel. The prepared metal salt solution was then added to the gel and stirred (by a mechanical stirrer) until the zinc and iron ions were uniformly distributed throughout the mentioned gel. Here, the container containing the sample was first placed at 90 °C for 24 hours for dehumidifying until a uniform, low-moisture, and faded brick red color gel was produced due to the formation of a set complex of iron ions and acetylacetonate. Then, the processed gel was placed in a muffle furnace at 550 °C for 4 hours. Since most of the gel is released as smoke, vapor, and foul-smelling gases up to about 300 °C, it is recommended that the previous step be performed under a fume hood and/ or laboratory ventilation. The obtained product was further treated (washed) with distilled water and ethanol. Next, an Ultrasonic Bath and a deionized water solvent were employed to disperse the nanoparticles and separate possible organic matter. Finally, ZIONC was dried in an oven at 75 °C for 24 hours after filtering the resulting mixture [23].

Characterization

At this stage, different techniques, such as Fourier-transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), scanning electron microscope (SEM), photoluminescence spectroscopy (PL), and transmission electron microscope (TEM), were employed toward characterizing synthesized ZIONC powder. Also, the size of the synthesized crystallite was estimated using the Debye-Scherrer equation through the following formula:

$$D = \frac{\kappa\lambda}{\beta\cos\theta} \quad (1)$$

In which:

D is the crystalline size in nm scale, λ is the wavelength of the X-rays (λ= 4 1.5416), k is the equilibrium constant of the equation and depends on the particle morphology (approximately equal to 0.89), β is the full-width at half-maximum (FWHM), and θ is the Bragg angle.

Injection of ZIONC

At first, normal saline was applied in preparing the investigated ZIONC suspension of the present study. Then, the diluted suspension was injected intraperitoneally using insulin syringes at doses of 1.25, 2.5, and 5 mg/kg/day for 12 days.

Studied animals (statistical community) and their care conditions

The study population of this study included 80 outbred adult male Wistar rats (220-250 g). The studied rats were kept in conditions with an average temperature of 25 °C, the relative humidity of 55 %, and a light/dark cycle of 12:12. It should be noted that the rats had easy access to standard food and water.

Sampling and sampling methods

In this study, 80 male rats were divided into eight groups (n= 10 per group) as follows:

1. The control group (CO) that did not take the ZION treatment and noise stress.
2. Rat groups (Three groups) that were treated with ZION at doses of 1.25, 2.5, and 5 mg/kg/d for 12 days while were not exposed to noise stress (CO+N1.25, CO+N2.5, and CO+N5).
3. Stress group (ST): Rats that were received normal saline and were exposed to noise stress for 12 days (from 8 AM to 12 o'clock for 2 hours).
4. Rat groups (Three groups) that were exposed to noise stress for 12 days (from 8 AM to 12 o'clock for 2 hours) and were received different doses of 1.25, 2.5, and 5 mg/kg/day at the beginning noise stress until the EPM test (ST+N1.25, ST+N2.5, and ST+N5).

Noise protocol

At first, the sound caused by traffic in one of the busiest squares of the Kashan city was recorded by a standard audio recorder to investigate the effects of noise stress in experimental animals. Then, its intensity was set equal to 95 decibels using sonar software (version 8.5). Next, a small speaker has placed at a distance of 30cm from the cage floor in the middle of the animal cage (a metal-reflective chamber with dimensions of 60 × 60 × 60 cm) to apply the recorded sound. Also, the sound intensity was monitored throughout the study using a sound level meter to apply noise

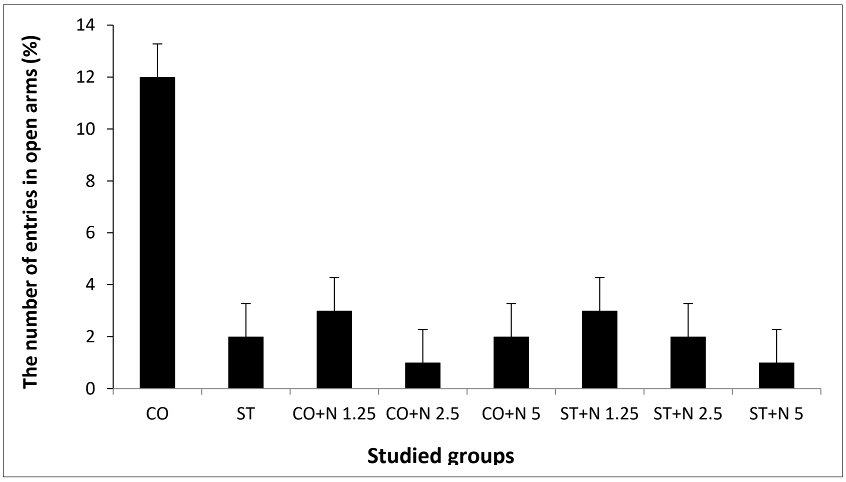

Fig. 1. The number of entries into open arms in the Elevated Plus Maze

stress with the same sound intensities according to the experimental treatments. On the other hand, although the recorded sound covered a wide range of audio frequencies, just annoying noises were considered here. Also, all study groups received sound (noise) stress at a specific hour (according to experimental treatments) every day [12].

Elevated Plus Maze Test

The basis of the Elevated Plus Maze (EPM) Animal Anxiety Test (EPM) is established based on the animal searching instinct and test animal's aversion to open and bright spaces. In other words, despite the animals' tendency to move and search in all arms, they avoid entering open arms. Accordingly, a strong approach-avoidance conflict is created for animals (conflict and anxiety are created in animals) and causes animals to spend most of their time in the closed arms [24].

The EPM is a metal or wooden plus (+) shaped maze with four black arms, which has a height of 50 cm above the ground. In general, two arms of the maze are open (in dimensions of 50×10 cm), and two other arms have walls of 40 cm in height without side and end roofs with dimensions of 50×10 cm. It was also placed a square in sizes of 10×10 cm at the intersection of the four arms. At this stage, animals were placed in a box with dimensions of 50×50×35 cm before every test for five minutes to familiarize the animal with the maze and increase the animal search activities. The selected rats were then placed in the maze room at least one hour until the test time. The animals were randomly selected and were then placed one after the other (facing one of the open arms) in the middle of the maze intersection square to move freely for five minutes. Eventually, it was evaluated the number of entries into closed and open arms and time spends on arms. In general, the numbers of entry or exit of rats' hands and legs were considered as inclusion and exclusion criteria for the studied evaluations, respectively.

The percentage of time spent in the open arms of the elevated plus-maze and the percentage of open arm entries for rats was calculated using the following formulas, respectively.

$$OAT\% = \frac{\mathrm{OAT}}{\mathrm{OAT} + \mathrm{CAT}} \times 100 \quad (2)$$

$$OAE\% = \frac{\mathrm{OAE}}{\mathrm{OAE} + \mathrm{CAE}} \times 100 \quad (3)$$

Where:

OAT, CAT, and OAE are Open Arm Time, Closed Arm Term, and Open Arms Entries, respectively.

Statistical analysis

The data were analyzed by SPSS (ver 20) and Two-way analysis of variance and Post hoc Tukey's test used at $p < 0.05$ to compare the mean of the data.

Ethical considerations

This paper has tried to perform ethical considerations in animal experiments by the ethical considerations of the Kashan University of Medical Sciences. Accordingly, the studied animals had access to adequate food and water, and the

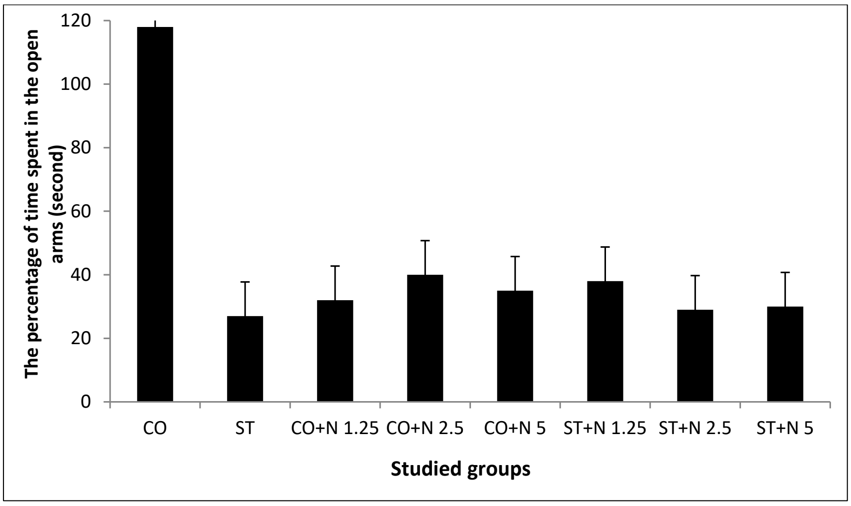

Fig. 2. The percentage of time spent in the open arms in the Elevated Plus Maze

lowest possible number of animals was also employed in this study.

RESULTS AND DISCUSSION

Investigating anxiety

The results of the Two-way analysis of variance and Post hoc Tukey's test showed that sound stress had significant effects on the parameters assessed between the control and the stress groups in rats in EPM (F7, 56= 3.123; P< 0.001).

Besides, it was observed that injections of ZIONC doses (1.25, 2.5, and 5 mg/kg/d) caused anxiety behavior in the control group so that behavior changes of rats in this group were similar to the rats' behavior in the stress group following the receiving the studied doses of ZIONC (P= 0.068, P =0.214 and P= 0.621, respectively). On the other hand, data analysis showed that injection of different doses of ZIONC (1.25, 2.5, and 5 mg/kg/day) had no significant effects on improving the anxiety behavior of nanocomposite-receiving stress groups (P= 0.187, P= 0.098, and P= 0.412, respectively).

The results of the obtained mean comparison between the application of different ZIONC doses to control and stress groups revealed that there were no significant differences in the parameters measured in the inhibitory avoidance task.

The number of entries of animals into open arms is shown in Fig. 2. According to the results, it was concluded that exposure of rats to sound stress led to adverse effects on their anxious behavior. In other words, there were significant and direct correlations between the frequency of entries into the open arms between control and sound stress groups. On the other hand, it was observed that injecting doses of 1.25, 2.5, and 5 mg/kg/day of ZIONC to the examined rats in the control group resulted in the incidence of anxiety. In this regard, the injection of all three studied doses of ZIONC had no significant effects on improving the anxiety behavior of animals in the stress group receiving nanocomposite.

The results of the data analysis of this experiment and the time spent of the tested animals in open arms indicated that sound stress caused anxious behaviors in rats so that the time spent of the animals in open arms in the sound stress group significantly shorter than the control group. On the other hand, although the injection of all three doses of ZIONC to the studied rats in the control group produced anxiety behavior, the injection of the mentioned doses had no significant effect on improving the behavior of rats in the stress group receiving ZIONC.

Results obtained from the synthesis of ZIONC

In the nanocomposite synthesis section of the present study, ZIONC was first synthesized and characterized according to the modified sol-gel technique. The obtained XRD pattern also showed that ZIONC was formed in three phases of $ZnFe_2O_4$, Fe_2O_3, Fe_3O_4 based on the standard picklists so that the mentioned phases created different peaks in 2Θ around 30, 35, 57, and 63° (Fig. 3).

The TEM image taken from ZIONC (see the

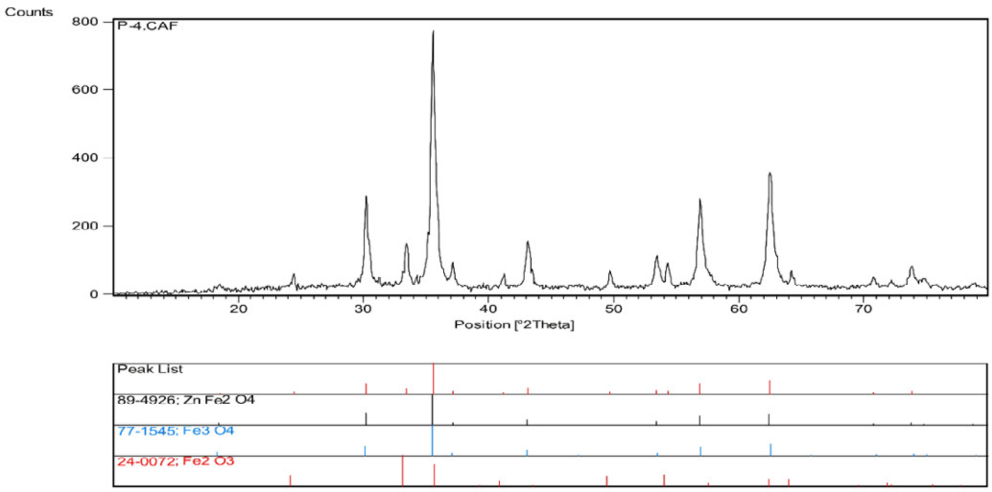

Fig. 3. XRD template for the synthesized ZIONC

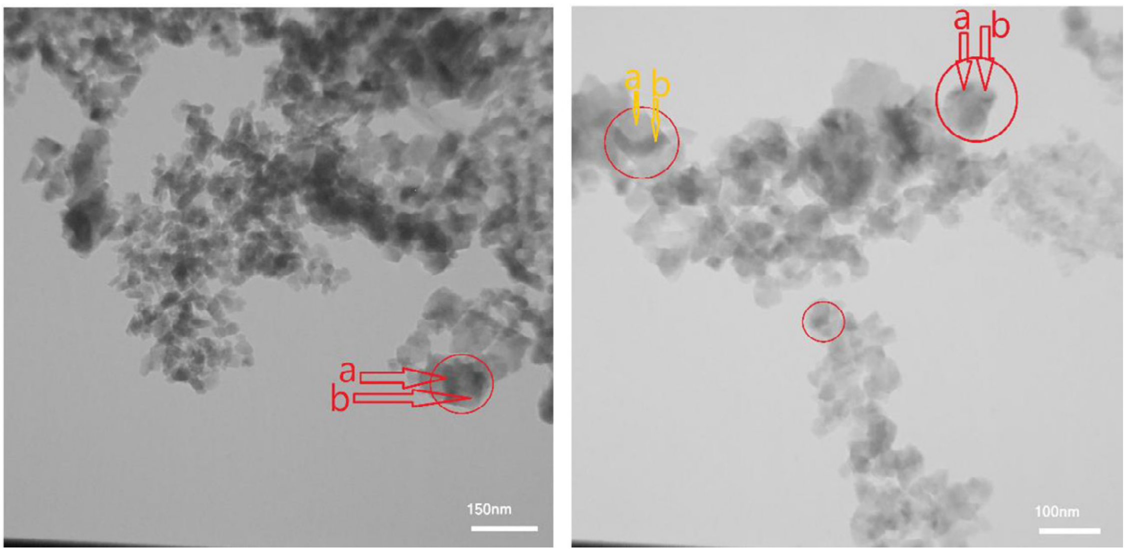

Fig. 4. Transmission electron microscope (TEM) image of the synthesized ZIONC

colored areas in Fig. 4) indicates the formation of a two-phase structure and a two-component composite derived from iron and zinc salts. Also, the synthesized plate-structured nanocomposite is observed in a range sizes of about 20 to 60 nm.

The plate-like structure of ZIONCs (synthesized by the green chemistry method) is clearly shown in the image obtained by the SEM technique. Since SEM is an analysis method to determine the morphology of particles' surface, Fig. 5 displays the contrast differences of the atomic number of the multiphase of the ZIONC, which confirms the formation of the composite structure of the synthesized product.

Investigating anxiety

Assessing the inhibitory avoidance behavior of animals using EPM is recognized as one of the most well-known approaches to investigate anxiety and anxiety-like behaviors in animal experiments. The statistical results of the present study confirmed that sound stress in EPM conditions had adverse

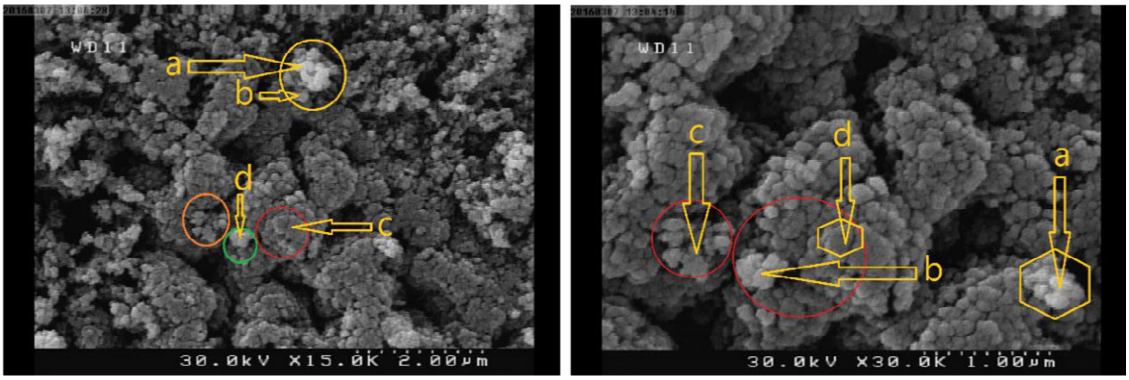

Fig. 5. Scanning electron microscope (SEM) image from the synthesized ZIONC

effects on the anxiety behavior of treated rats so that, considering the measured parameters, significant differences were observed between the control and stress groups.

In contrast, the injection of the different doses of ZIONC induced anxiety in both control and stress groups, and animals in the two groups showed the same behaviors. Statistical analysis of the data also determined that the injection of different doses of ZIONC had no significant effects on improving the anxious behavior in the noise-exposed animals. On the other hand, the data taken from the ZIONC treated animals indicated significant differences between the control and stress groups.

Our findings indicated the destructive effects of sound stress on the anxiety behavior of the testing rats. Some studies have shown that reducing the intensity of acoustic waves decreased stress hormones, especially norepinephrine. Therefore, through stimulation of the hypothalamus-pituitary-adrenal axis (HPA), stress stimulates the release of the cortisol and corticosterone hormones in humans and rodents, respectively. Prolonged exposure to acoustic waves leads to physiological and psychological disorders and increased oxidative stress [25]. Ahmadi *et al*. (2017) found that different levels of noise stress increased the anxious behavior in the treated animals so that the number of entries into and time spent in open arms was decreased in the EPM [25]. Consistent with our results one study reported increased anxiety behavior in rats exposed to sound stress [26].

Also, the application of ZIONC in control groups resulted in increasing anxiety and decreasing the number of entries into the open arms and time spent in open arms. Torabi et al. (2015) reported that administration of 1 mg/kg of zinc oxide and zinc nano-oxide (ZnO NP) significantly reduced the anxiety behavior of rats. Also, they showed that the anti-anxiety effects of ZnO NPs were higher than those of zinc oxide. Therefore, the anti-anxiety effects of ZnO NPs may be due to the physical and chemical properties of nanostructures and their small size, which leads to increased strength and penetration rate into the body tissues. It has also been confirmed that a high surface area to volume ratio in nanoparticles increases the availability of active-sites than the conventional compounds and can consequently have greater effects in smaller amounts than similar conventional compounds [27]. In general, it can be stated that zinc ions are involved in the stimulation and activity of several enzymes in the body and affect receptors of numerous neurotransmitters such as GABA, Serotonin, NMDA, and voltage-gated calcium channel [28]. These receptors can also play principal roles in the modulation of anxiety. Besides, since zinc ion is an essential element for the physiological function of the brain and other organs [29], the use of organic and inorganic supplements containing zinc can partially improve anxiety in animal models [30]. Despite the limited information on the mechanism of the anxiolytic effects of ZnO NPs, it has been suggested that zinc ions released from nanocomposites may have anxiolytic effects in animals via at least two mechanisms [28]. First, some studies have shown that zinc ions regulate glutamate signaling by inhibiting NMDA receptors. In general, zinc ions and glutamate are released together in the presynaptic space. Glutamate is one of the most important excitatory neurotransmitters in the modulation of anxiety-like behaviors. Zinc ions impair glutamate function in anxiety by inhibiting the most well-known glutamate receptor or NMDA receptor. Second, zinc ion acts as an inhibitory neurotransmitter reducing glutamate presynaptic output, decreasing glutamate signaling, and subsequently preventing the anxiety occurrence through increasing GABA presynaptic output [31].

Khajehpour et al. (2019) reported that despite the additive effects of intraperitoneal injection of ZnO NPs, with Fe2O3 phase, under a dose of 7.5 mg/kg on the anxiety behavior of adult male rats, it was observed no significant effects on changing the anxiety behaviors of rats treated with a concentration of 5 mg/kg of ZnO NPs. They attributed the results to iron deficiency, especially in the dorsal hippocampus, which may induce increased anxiety-like behaviors in animals. It has also been suggested that high levels of iron in the brain can lead to decreased brain function, increased neuronal death, and impaired production and release of neurotransmitters including acetylcholine, adrenaline, and serotonin. Also, abnormal functioning of these systems can cause increased anxiety behaviors [31].

Kesmati and Khorshidi (2013) also found that intraperitoneal injection of 0.2 mg/kg of Fe_2O_3 nanoparticles into rats had no notable effects on animal anxiety behaviors while injections of 2 and 5 mg/kg reduced the anxious behaviors of the studied animals. Their results were due to the anti-anxiety properties of Fe_2O_3 nanoparticles at doses of 2 and 5 mg/kg [16].

Different researches focus on iron oxide nanoparticles. In one study, intraperitoneal injection of Fe_2O_3 nanoparticle with a concentration of more than 7 mg/kg had synergistic effects on anxiety behavior while application of 5 mg/kg level had no significant influences on this parameter. On the other hand, doses of 2 and 5 mg/kg of Fe_2O_3 had anti-anxiety effects in another study. In general, in previous studies, iron seems to be functional in the ferric (Fe^{3+}) state, but the ferrous (Fe^{2+}) state is present in a part of the stoichiometry of the preparation of ZIONC. Intraperitoneal injection of ZIONC helps zinc ions to transmit through the bloodstream to other organs of the body. Also, due to the presence of the ferrous state of the iron element in red blood cells, the increase in Fe^{2+} levels in the blood can appear via the ionic transport of the synthesized ZIONC. According to a study by Eseh and Zimmerberg (2005), iron deficiency leads to an increase in anxiety behavior in female rats [32]. Hence, it can be concluded that increasing the amount of Fe^{2+} state leads to improved anxiety behavior in animals.

On the other hand, since serotonin plays critical roles in mediating emotional behaviors in the brain, iron deficiency causes changes in the serotonergic system that lead to the incidence of anxious behaviors. However, the effect of iron deficiency in the dopaminergic system is more durable and prevalent (which has both effects on increasing anxiety and changing motor activity). More than anything, chronic (long-term) iron overload can also damage a wide range of body tissues, including beta cells in the liver, heart, and brain. Iron accumulation increases the incidence of diastolic and systolic heart failures, which in turn impairs cardiac function, increases oxidative stress, and Intensify anxiety. On the other hand, iron overload can lead to an increase in reactive oxygen species (ROS), disrupt in the function of antioxidant (AO) defense systems, and consequently increase oxidative stresses. Despite many studies in this field, there is no proven mechanism on how iron-overload affects and disrupts the antioxidant systems. High levels or deficiency of iron seems to affect oxidation and reduction mechanisms and affect the occurrence of anxiety behaviors through affecting the antioxidant defense systems and changes in the concentration of the produced oxidants. In general, it is proven that disturbances in iron concentrations in various brain regions, including the hippocampus, create significant effects on the occurrence and development of anxiety behaviors [33], which is consistent with the observed effects of ZIONC on anxiety-like behaviors in the present study. Because of the highest concentrations of iron in brain regions, such as dopaminergic structures, the indirect effects of iron on anxiety and pain behaviors mediated have been investigated by the dopaminergic system in some studies [34]. It has also been reported that iron deficiency-induced in the rats' diet leads to a decrease in dopaminergic receptors [35] [25, 26]. Besides, anxiety-like behaviors were found to be associated with density and dopamine receptor D2 in the prefrontal cortex. Accordingly, the iron has a direct role in the synthesis and binding of dopamine receptor D2, and its reduction can reduce dopamine receptors in the striatum and midbrain [26]. Given the above, the iron released from ZIONC may exert a part of its anti-anxiety effect through the dopamine system. On the other hand, identifying systems or mechanisms that mediate this process requires more detailed future studies.

Synthesis of ZIONC

In general, the obtained results of the XRD analysis showed that the intensity of the peaks of the synthesized ZIONC was lower than the peak intensity for the bulk state analysis. The expanding of the base of the peaks indicates nanoscale structures for the synthesized ZIONC. The size of the synthesized crystallite was estimated equal to 20 nm using the Debye-Scherrer equation. Also, a comparison of the obtained XRD pattern with standard picklists and previous works for different pattern peaks in 2Ɵs around 30, 35, 42, and 58° showed that ZIONC was formed in the ZnFe2O4 phase [36]. Here, the characterization of the synthesized ZIONC surface was detected using SEM images [5]. ZIONC disk and plate structures are clearly defined in the obtained SEM images. According to the obtained a, b, c, and d regions in Fig. 4 and the contrast of the atomic number (Z-contrast), it can be observed that the brightest and darkest phases indicated the highest and lowest atomic numbers, respectively. Hence, the above results confirm the multiphase structure of the XRD pattern. According to the nanostructured synthesis technique and raw materials used in the present study, a maximum of four phases $ZnFe_2O_4$, Fe_3O_4, Fe_2O_3, and ZnO were identified with molecular masses of 241, 231, and 159 g/mol. On the other hand, it was found that regions α and b are closer to the ZnFe2O4 phase through examining the XRD pattern and the proximity of the molar masses of the two phases of $ZnFe_2O_4$ and Fe_3O_4 with the numbers of 241 and 231. Besides, regions c and d may correspond to the Fe_2O_3 and ZnO phases. Therefore, according to the above evidence, it can be recognized that the powder synthesized by the modified cell-gel technique is a multiphase nanocomposite consisting of zinc and iron oxides.

TEM images are a well-known analysis in determining the structure of nanocomposites. The amalgamation of different phases of nanocomposite formations, as a primary condition of a nanostructure formation, is detected by the electron beam passage from the structure of nanoparticles and nanopowders. The results in Fig. 4 (Atomic Number Contrast Technique) showed that at least two mixed phases were formed in regions α and b.

The standard sol-gel process is a wet-chemical technique that uses solvents, such as ethanol and molecular precursors (usually metal alkoxide), toward synthesizing various types of nanostructures, especially metal oxide nanoparticles. In this study, natural gelatin was applied as a gelling and coating agent. Also, water was used as a solvent, and simple mineral salts were used as precursors by removing expensive precursors of the Alkoxy group and alcohol solvent. Therefore, the production of wastage in this method is reduced, which is known as an indicator in green chemistry and environmentally friendly chemical reactions, and less damage to the environment. Also, the speed of the process of converting sol to gel and, thus, the compaction speed of gel encourage using this method.

Laser Ablation Nanofilms technique is a proper method in the synthesis of different composites of zinc and iron oxides [37]. Also, it is shown that Fe-ZnO nanocomposites are prepared using metal nitrate precursors by the application of coprecipitation and sonochemical methods [38, 39]. Besides, the Fe_3O_4/ C/ ZnO three-component nanocomposite was obtained in some studies by a one-step sol-gel process, in which lignin amine (LA) was utilized as the carbon source and ligand donor agent [39].

Various mixtures of oxide nanocomposites, such as $Fe_2O_3/ZnFe_2O_4$, $Fe_2O_3/ZnFe_2O_4$/ZnO, and $ZnFe_2O_4$/Zn, were formed by a hydrothermal method via changing the molar ratios of iron and zinc salts and then calcination at 500 °C [40]. In some studies, the coprecipitation technique was also employed to synthesize ZIONC using natural animal gelatin (pig skin gelatin) as a capping and ligand donor agent [41].

CONCLUSION

Sound stress profoundly affects anxiety and anxiety-like behaviors. The results of the present study showed that the intraperitoneal injection of ZIONC in sound stress rats reduced anxiety-like behaviors. Also, the modified sol-gel (MSG) method and using gelatin in ZIONC synthesis led to the production of plate-structured nanocomposites.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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