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Anti-anxiety and antioxidant properties of the zinc nanoparticles green-formulated by an ethnomedicinal plant in the in vivo condition

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Abstract

Objective: Recently, the medicinal plants green-synthesized metallic nanoparticles have been used due to the therapeutic effects such as neuroprotective properties. In the recent study, the application of zinc nanoparticles containing *Ocimum basilicum* leaf aqueous extract on anxiety in male mice was investigated. The results of analyzing the absorption spectrum of zinc nanoparticles by FT-IR method showed that these nanoparticles show absorption peaks at wavelengths of 3448, 2955, 1624, 1040 and 539, that these peaks are related to O-H, C-H, C=O, C-O and Zn-O bonds in these nanoparticles, respectively. The results of examining the antioxidant properties of zinc nanoparticles in the DPPH free radical test revealed that the nanoparticles have excellent antioxidant effects against the DPPH free radical. ZnNPs green-synthesized by *O. basilicum* could reduce the anxiety in male mice at the Light/dark transition test. After doing the clinical trial studies, the recent nanoparticles may be used as a neuroprotective supplement in humans.

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Introduction

Currently, neurological disorders are one of the most common disorders in the whole world and especially in Iran. Electrical, biochemical, or structural disturbances in the spinal cord, brain, or other nerves can lead to a symptoms wide range [1]. Symptoms examples include cognitive impairment, pain, confusion, seizures, sensation loss, poor coordination, muscle weakness, and paralysis. There are many known neurological disorders, some are fairly usual, but very rare. They may be examined by neurological treated and examination in the specialties of neurology and clinical psychology. Many drugs that are initially used to treat nervous disorders include psychoactive drugs such as amphetamine, nicotine, tramadol, etc. Excessive use of the above drugs causes the exacerbation of neurological disorders, so that after a while, the patient must use very high doses and much stronger drugs [2]. Nicotine is one of the most important psychoactive drugs. Nicotine is an alkaloid extracted from the dried leaves of the tobacco plant (Nicotiana tobacum). Millions of people around the world are exposed to it through smoking or inhaling insecticides. Physically, nicotine is an oily and water-soluble substance [3]. Nicotine is a base in water and as a nitrogenous base with acids, it forms salts that are usually solid and soluble in water [4]. The release of nicotine burns at a temperature of 95°C, and because of this, most of the nicotine in the cigarette is burned and smoked; However, the same small amount can cause addiction. Nicotine is easily absorbed through the skin, and considering its solubility in water, one of the ways to obtain it is to soak chopped tobacco in water for 12 hours [4,5]. By affecting the central and peripheral nervous centers, nicotine causes changes in cardiovascular, nervous and endocrine actions. Nicotine works as a nicotinic acetylcholine receptor agonist [2]. In small amounts, this substance stimulates these receptors and increases the secretion of adrenaline hormone, which causes an increase in blood pressure, heart rate, breathing, and finally, an increase in blood glucose. Nicotine in larger amounts causes the depolarization of nicotinic acetylcholine receptors, which is one of the reasons for nicotine's toxicity, and this property is used as an insecticide [5]. Various chemical drugs are used to treat high doses of nicotine to prevent neurological disorders. Most chemical treatments cause several side effects, including weight change, appetite loss, throat and mouth burning, dental and gum problems, vomiting and nausea, fatigue and depression. The high prevalence of mental disorders and the ineffectiveness of chemical treatments indicate the need to find natural and new medicinal compounds that have fewer side effects [3,5].

Iran's strong dependence on the import of pharmaceutical raw materials and the withdrawal of large amounts of foreign currency from the export of crude oil reveal the need to pay serious attention to the talents and abilities in the fields of processing, production and exploitation of herbal medicinal products [6-9]. Also, because of the long history in herbal therapy and traditional medicine, this is felt more than ever. For this reason, and in order to reduce or avoid the adverse effects caused by the raising chemical drugs use, the global trend and public approach to the use of medicinal plants is increasing. Studies have shown that some types of compounds found in plants that are prepared in a pure form in the laboratory, if they are used together with other compounds found in the plant, their side effects disappear and only its beneficial effects are revealed in the person [10-12]. The knowledge of medicinal plants and their compounds industrial or medicinal use have been the focus of many scientists since ancient times. Basil (*Ocimum basilicum*) from the mint family has long been considered as a medicinal plant in the treatment of many diseases [13-15]. With the investigations, the essential oil of this plant and especially the phenylpropanoid compounds are known as the main medicinal compounds of this plant. Basil is used traditionally and natively in different regions of Iran. Normally, applications such as preventing heart attack, preventing depression, increasing sperm production, preventing nausea, increasing breast milk and creating a feeling of joy and happiness can be seen in different regions of Iran [15-17]. But in particular, we can mention areas such as Kazaron in Fars province, where this plant is used to heal mouth sores and reduce fever. In Manalaqa of Dashtestan (Bushehr province), basil is known as a stomach-strengthening, anti-depressant and anti-inflammatory sedative and is used by the people of those areas [17,18]. Recently, the metallic nanoparticles have been used for increasing the therapeutic effects of the plants. One of these nanoparticles is zinc nanoparticle. Zn nanoparticles have been used for the treatment of several diseases such as cancers, bacterial and fungal infections and nervous disorders.

In the recent study, the application of zinc nanoparticles containing *Ocimum basilicum* leaf aqueous extract on anxiety in male mice was investigated.

Methods and Materials

The ethical committee code was IR.BMSU.REC.1399.012 for the recent study.

Preparation of extract

After completely drying the basil leaves in a dark place without humidity for a week, the intended substance was powdered. 200 gr of plant powder was carefully weighed and 2000 ml of distilled water was poured on it (at a ratio of one to ten weight/volume), the mixture was heated for 2 hours at a temperature of 40 degrees Celsius and stirred at the same time. Then, it was left at room temperature for 24 hours and after the corresponding time, the extract was filtered by Whatman No. 2 paper. The primary extract entered the rotary with vacuum pump and the solvent was evaporated at a temperature of 80 degrees Celsius and for one hour. In this way, the concentrated extract was obtained [19].

Preparation of zinc nanoparticles

For the synthesis of 2 grams of zinc oxide bio-nanoparticles, 6.94 gr of zinc nitrate $[Zn(NO_3)_2.6H_2O]$ were poured into 50 ml of distilled water while stirring after weighing. Then add 50 ml of water extract of the plant (500 gr/l) until the total volume of the solution reaches 100 ml. The pH of the solution was adjusted to 10 with 5 M NaOH. Then the resulting solution was placed in a microwave (2.45 GHz and 1000 W) for 10 minutes. The resulting precipitate was centrifuged for 5 minutes at 3000 rpm (manufactured by Applied Biosystem, USA) and washed once with distilled water and then with ethanol and centrifuged. Finally, the samples were placed in an oven at a temperature of 60 degrees Celsius for 24 hours to dry, and then stored in powder form for the next step.

Measuring the antioxidant activity with DPPH

DPPH test (Germany, Merck) is used to check the antioxidant activities of different compounds. DPPH in ethanol turns into its radical form, which has the highest absorbance at the wavelength of 517 nm. This radical reacts with the antioxidant substance and is removed. So, its absorption reduces at the wavelength of 517 nm. To prepare the DPPH, 2 mg DPPH was dissolved in 33.8 ml ethanol. Several dilations of zinc nanoparticles were prepared and mixed with an equal proportion of DPPH solution. This solution thoroughly was mixed by vortexing for 10 sec and kept in the dark for 30 min at room temperature. In this test, butyl hydroxytoluene BHT (Germany, Merck) was used as a positive control. This experiment was performed three times and the following formula was applied to measure the percentage of DPPH radical inhibition.

$$AA\% = \left[\frac{A0 - A1}{A0}\right] \times 100$$

In relation, A0 is the absorption of negative control (DPPH) and A1 is the absorption of zinc nanoparticles and hydroxytoluene butylate [19].

Measuring the anti-anxiety activity of zinc nanoparticles *in vivo*

56 adult and healthy male BALB-c mice were selected from Kermanshah University of Medical Sciences. In terms of physical conditions, the animal housing had a light and dark period of 12 hours, a temperature of 23-35°C and a relative

humidity of 40-60% without noise pollution. Animals were fed with special rat food. In order to create a compromise with the environment, the mentioned conditions were controlled for 15 days. At the beginning of the experiment, mice were randomly divided into 7 equal groups as follows;

1) Control group: received 0.2 ml of distilled water daily for thirty days.

2) The first treatment group: for thirty days, mice received 0.2 ml of $Zn(NO_3)_2.6H_2O$ (0.5 mg/kg).

3) The second treatment group: for thirty days, mice received 0.2 ml of $Zn(NO_3)_2.6H_2O$ (1 mg/kg).

4) The third treatment group: for thirty days, mice received 0.2 ml of basil plant aqueous extract (0.5 mg/kg).

5) The fourth treatment group: for thirty days, mice received 0.2 ml of basil plant aqueous extract (1 mg/kg).

6) The fifth treatment group: for thirty days, mice received 0.2 ml of zinc nanoparticles in the aqueous extract of basil (0.5 mg/kg).

7) The sixth treatment group: for thirty days, mice received 0.2 ml of zinc nanoparticles in aqueous extract of basil plant (1 mg/kg).

On days 5, 10, 15, 20, 25, 30, using the Light/Dark Transition Test, the level of anxiety in control and treated mice was measured. In this way, the times spent in the dark area indicated the absence of anxiety and the times spent in the light area indicate the anxiety of the studied mice [19].

Statistical analysis

In order to analyze the results obtained in this study, ANOVA and T-test were used using SPSS software. In all cases, P less than and equal to 0.01 was considered as a significant difference.

Results and Discussion

The results of analyzing the absorption spectrum of zinc nanoparticles by FT-IR method showed that these nanoparticles show absorption peaks at wavelengths of 3448, 2955, 1624, 1040 and 539, that these peaks are related to O-H, C-H, C=O, C-O and Zn-O bonds in these nanoparticles, respectively (Figure 1).

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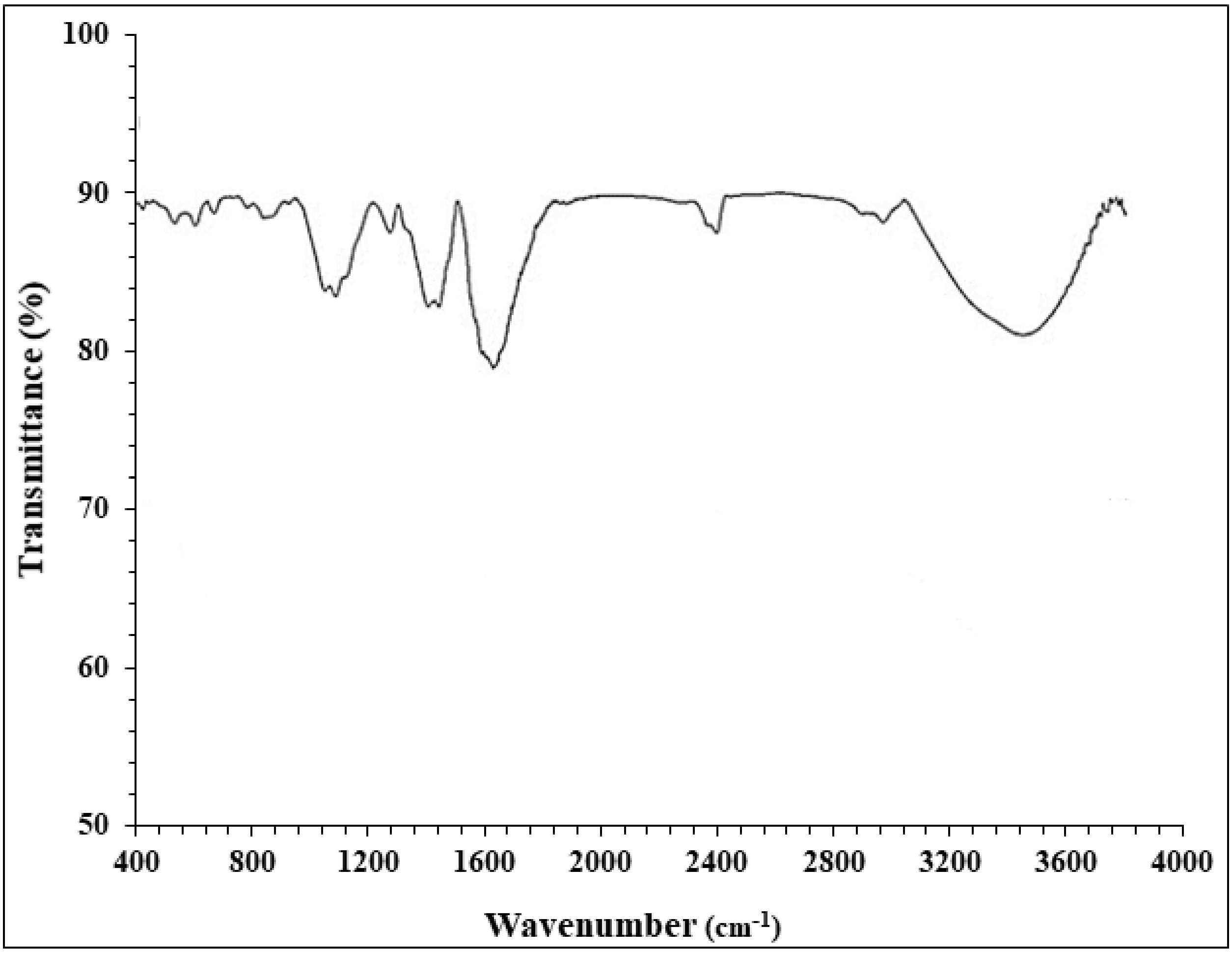

Figure 1. FT-IR pattern of Zn nanoparticles.

Antioxidants are related in the prevention of degenerative diseases such as cardiovascular, neurological, cancer and oxidative stress disorders [20]. Basil aqueous extract has good antioxidant activity. Also, studies have shown that this plant has significant antibacterial properties and therefore can be used in food and pharmaceutical industries. The aqueous extract of basil also has the highest antimicrobial effect on the GP bacteria Staphylococcus epidermis and the least antimicrobial effect on the GN bacteria Enterobacter aeruginosa. Because of the beneficial antioxidant's effects, interest in finding natural antioxidants has increased. Medicinal plants with their antioxidant activities are also useful for preventing cardiovascular diseases by reducing lipid peroxidation [20,21].

The antiradical activity of plants can be attributed to the presence of oxygenated monoterpenes such as linalool and linalyl acetate, in such a way that if the concentration of these substances increases in plants, the amount of inhibitory activity also increases in them [22]. On the other hand, these compounds have antioxidant synergistic properties with other substances present in the essential oils of plants. For example, linalool and eugenol together have synergistic antioxidant properties and also have an effect on other active compounds and increase their activity. Basil's strong action against free radicals can be attributed to the presence of these two substances. Basically, with the increase of total phenolic compounds, their antioxidant property also increases [23-25]. Hashemi et al. [26] reported that the maximum amount of antioxidant activity in basil plant is under the effect of extract extraction by ultrasonic method and temperature of 45 degrees Celsius. In this research, the maximum amount of this activity in the basil plant was also under the effect of extract extraction by ultrasonic method. Sami et al. [27] reported the rate of free radical inhibition of basil

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plant as 31.2%. They also stated that the higher the amount of phenolic and flavonoid compounds of a plant, the higher their antioxidant activity will be. Juliani et al. [28] reported that the amount of antioxidant activity in purple basil is higher than that of green basil. Their investigations showed that the amount of this activity has a direct relationship with the amount of phenolic and flavonoid compounds.

The results of examining the antioxidant properties of zinc nanoparticles in the DPPH free radical test showed that the nanoparticles have excellent antioxidant effects against the DPPH free radical. In addition, with the increase in the concentration of the mentioned substances, the inhibition percentage of free radicals increased significantly (Figure 2).

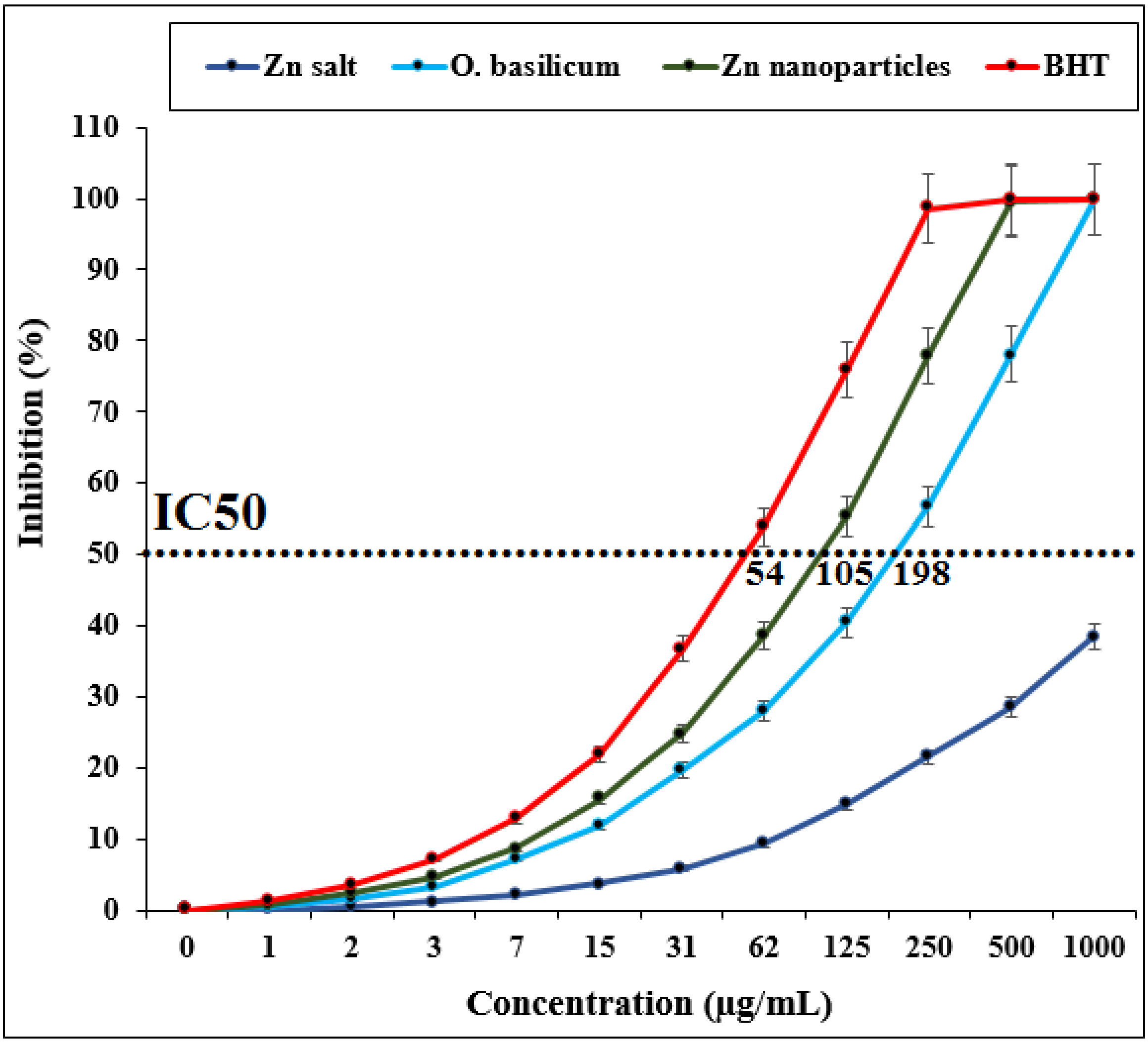

Figure 2. Antioxidant effects of zinc nanoparticles salt in DPPH free radical test.

The results of *in vivo* study show that zinc nanoparticles and basil extract in both doses caused a significant decrease in anxiety compared to the control group and zinc salt treatment groups by reducing the remaining mice in the light section ($p \geq 0.01$). There is no significant difference in the control group and zinc salt treatment groups ($p \geq 0.01$) (Figure 3).

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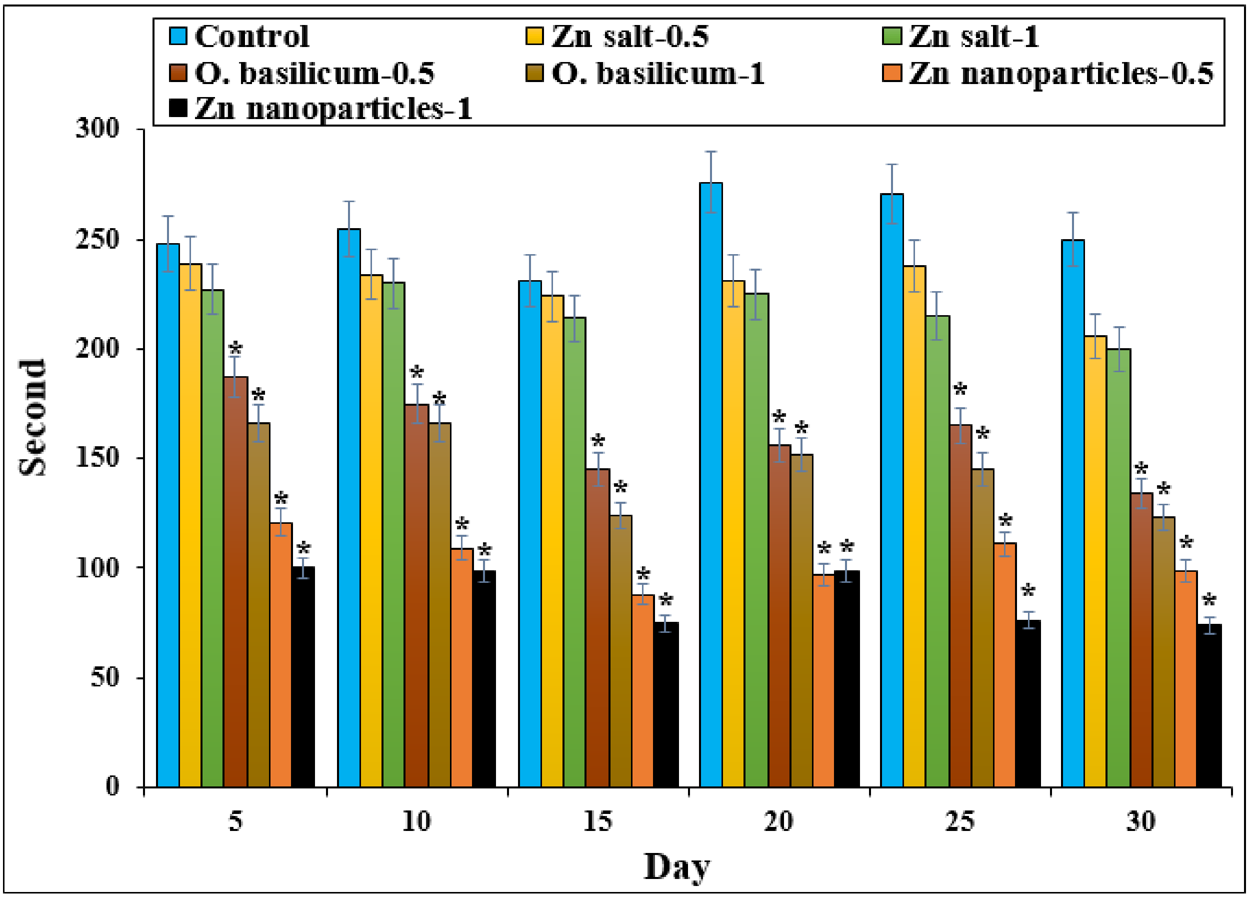

Figure 3. The effect of nanoparticles on the survival rate of mice in the light part of the anxiety test (Light/dark transition test).

* Indicates a significant difference between the nicotine treated group and other treated groups (p≥0.01).

The results show that zinc nanoparticles and basil extract in both doses caused a significant decrease in anxiety compared to the control

There is no significant difference in the control group and zinc salt treatment groups ($p \geq 0.01$) (Figure 4).

group and the treatment groups with zinc salt by increasing the remaining mice in the dark section ($p \geq 0.01$) (Figure 4).

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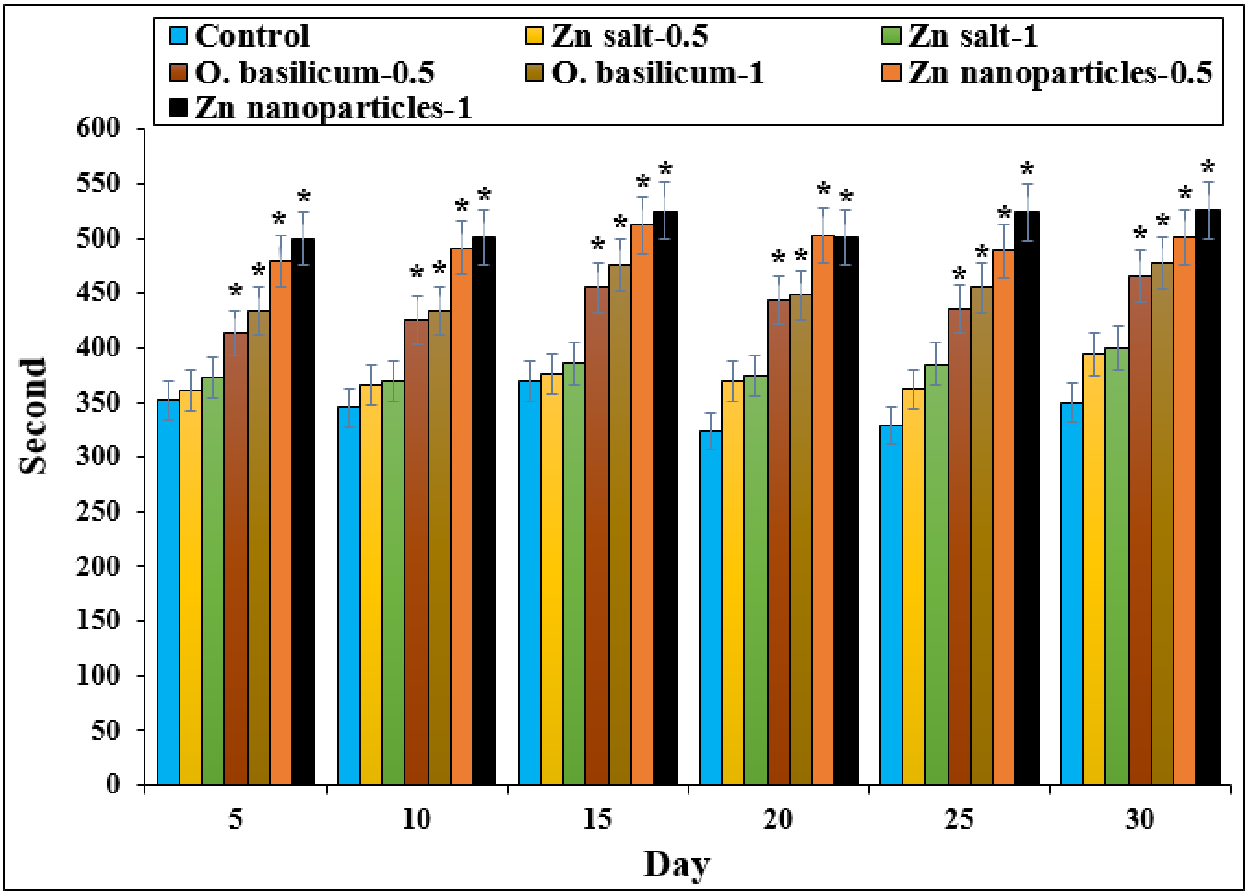

Figure 4. The effect of nanoparticles on the survival rate of mice in the dark part of the anxiety test (Light/dark transition test).

* Indicates a significant difference between the nicotine treated group and other treated groups (p≥0.01).

Drug delivery to the CNS is a challenge faced by biologists because of protective barriers. The drug effectiveness in the CNS depends on the drug ability to cross the blood-brain barrier and reach therapeutic concentrations in the brain after administration [29]. Therefore, failure in the treatment of CNS disorders is often not because of a drug potency lack, but due to a problem in the method of drug delivery. Nanotechnology, especially the use of nanoparticles, has registered promising answers [30]. Generally, pharmacotherapy must have a unique shelf life in the blood to be effective. Nanoparticles are usually more effective because of their small size. Several factors such as surface characteristics, surface charge, weight, shape and size are effective in the nanoparticles persistence in the blood [31,32]. The low volume-to-surface ratio of nanoparticles gives them the ability to bind multiple ligands to their surface [33]. In another study, neuron-protective peptides were placed in nanostructures of monosialotetrahexosylgan glyoside for the Alzheimer's disease combined treatment [34]. Also, nanoparticles coated with polyethylene or polysorbate and with a size smaller than 100 nm can be protected from the reticuloendothelial system. Nowadays, because of the presence of negative charges on the endothelial cells surface, it is possible to create electrostatic interaction by functionalizing the nanoparticles surface with positively charged biomolecules. As a result, the nanoparticles passage through the blood-brain barrier is facilitated. Nanoparticles pass through

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the blood-brain barrier through transferrin and lipoprotein receptors inside the cell through absorption [35]. For example, cell harvesting starts with binding of transferrin to its receptor and continues with endocytosis, which can pass the mixture of drugs and nanoparticles through the blood-brain barrier [36]. Also, the modified nanoparticles circulation in the blood causes easier interaction and penetration into the endothelial cells and the possibility of more control in the actions of the cells. However, despite the current progress in the science of nanomedicine, the use of nanoparticles still faces problems, for example, unknown tissue unpredictable and interactions consequences [31,37]. Cationic Au nanoparticles enter the cell without consuming energy and by bypassing

The small size of NPs reduces the ratio of volume to surface and as a result it can absorb more free radicals and in this way it can be a suitable solution to deal with free radicals released after nerve damage [42]. Soluki et al determined the effects of cerium oxide nanoparticles on improving motor performance and tissue changes following sciatic nerve injury in rats. The speed of the repair process and improvement of motor function in the groups treated with cerium oxide increased significantly compared to the control group [43]. Also, in another study that investigated the oxidative stress induced on nerve cells and endothelial cells, it was found that cerium oxide NPs reduced cell apoptosis and were effective in repairing peripheral nerves [44]. Also, the effect of this substance in angiogenesis, modulating the nervous system, anti-cancer applications, inhibiting high blood pressure, antibacterial effects, reducing cholesterol levels, reducing damage/damaged tissue has been confirmed [43,44]. Because of the properties of nanoparticles, there is great hope for creating treatment and diagnostic facilities in medicine. One of the prominent molecules for therapeutic and diagnostic applications is magnetic nanoparticles, which can move drugs to preferred locations by a magnetic field [114]. MNPs prevent the accumulation and inflammation of damaged tissue, and are also used as biomarkers to assess drug effectiveness. methods and affect the function of the cell [39]. Nanomaterials can be applied as antioxidant enzymes carriers. Antioxidant enzymes are capable of reducing ROS, but they remain in the blood for a short period of time and then decompose. Therefore, it is hard to cross the blood-brain barrier because of their short presence [40]. Most of the drug delivery systems are in the polymer nanoparticles form, these nanoparticles have the ability to pass through tight cell junctions. They also have a high drug loading capacity and raise the combined drug's effectiveness [41]. In this regard, nanospheres and nanocapsules are vital in new drug delivery, because they have a high drug loading capacity. Therefore, they increase the probability of the drug reaching the brain [42].

By effectively delivering growth factors and stimulating regeneration, the magnetic nanoparticles role in peripheral nerve repair has been proved [45,46]. Scientists also indicated that the neurotrophic factors covalent combination with iron oxide nanoparticles raised the stability and improvement of peripheral nerves [46]. The researchers found that the sciatic nerve grafting with channels designed with nanocomposites was successful, and the animals whose sciatic nerve was grafted with this method showed a higher nerve message conduction speed than other groups. [47]. It was also observed in a research that the presence of zinc nanoparticles in the matrix intelligently increases the proliferation of nerve cells due to their conductivity and biodegradability [48]. Scientists are trying to repair nerves by optimizing the properties of nanoparticles and stimulation parameters. Gold nanoparticles, as a substrate and matrix that is electrically conductive, are a promising material for the regeneration of peripheral nerves [45-47]. In the diseases of the nervous system, it is possible to disperse very small medicinal particles in an external liquid phase by using nanosuspensions. Ease of manufacturing process, much lower toxicity and increased efficiency are the advantages of nanosuspensions [46,47]. Nanocarbon formulation can also be used for various applications such as cancer diagnosis,

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imaging and drug delivery and tissue engineering [45-48].

Conclusion

In this study, zinc nanoparticles were synthesized using basil leaf aqueous extract and $Zn(NO_3)_2.6H_2O$. To identify the chemical characteristics and morphology of nanoparticles, FTIR analyzes were used. The results of analyzing the absorption spectrum of zinc nanoparticles by FT-IR method showed that these nanoparticles show absorption peaks at wavelengths of 3448, 2955, 1624, 1040 and 539, that these peaks are related to O-H, C-H, C=O, C-O and Zn-O bonds in these nanoparticles, respectively. In order to investigate the antioxidant effect of nanoparticles, the DPPH method was used. The results of examining the antioxidant properties of zinc nanoparticles in the DPPH free radical test showed that the nanoparticles have excellent antioxidant effects against the DPPH free radical. Also, zinc nanoparticles were studied to reduce anxiety in mice. After conducting clinical trial studies, zinc nanoparticles synthesized with basil leaf aqueous extract can be used as a protective therapeutic supplement for neurotoxicity.

Authors' contribution

All authors contributed equally to the manuscript.

Conflicts of interest

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication and etc.) have been completely observed by author.

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