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Sports injury treatment and sports rehabilitation employing the Nanoparticles containing zinc oxide

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Abstract. The combination of physical activities and individual skills in sports creates an entertaining and competitive environment governed by a set of rules. In today's world, sports attract significant attention and are approached differently by various groups. Inevitably, injuries occur in sports, significantly impacting an athlete's performance and ability to participate in exercises and competitions. Addressing this issue, one of the crucial measures involves restoring the athlete's ability to engage in sports and compete. Sports rehabilitation serves as a treatment to mitigate the effects of injuries, and when combined with surgery, it can expedite the recovery process. Therefore, the primary objective of this study is to utilize a biocompatible technology for synthesizing zinc oxide (ZnO) nanoparticles in sports rehabilitation, ensuring minimal harm to the environment.
Keywords: athlete; injuries; nanoparticles; rehabilitation; sports

1. Introduction

Routine physical activity is one of the necessary actions anyone can take to maintain and improve their health (Verboven and Hansen 2021). The health usefulness of regular exercise and physical activity for the body and heart cannot be ignored (Omidi *et al*. 2013, Ghadiri *et al*. 2016c, Mousavi *et al*. 2017). Sports is one of the issues discussed worldwide, and many groups deal with it differently (Prado *et al*. 2022). The importance of sports, paying attention to sports, and trying to develop them among all strata of society is due to the beneficial effects that can be obtained from this work because sports prevent unemployment, fight against boredom and laziness, and increase physical and mental abilities (Xu 2022). Courage is physical and mental health and a means for collectivism and socialization (Shafiei and She 2018, Shafiei *et al*. 2019, Shafiei *et al*. 2020). The personal effects of sports affect various aspects of a person. Sports movements, on the one hand, increase the athlete's strength and physical ability, on the other hand, they make the human body healthy (Nieman and Wentz 2019, Heilmann *et al*. 2022). Exercise can be considered a natural stimulus for defense cells in teenagers and adults. In older people, the function of the defense system weakens itself, which can increase the risk of infectious and contagious diseases (Karacabey 2005). Nevertheless, regular and balanced sports exercises such as walking and cycling can reduce this weakness of the defense system in the body (Azimi *et al*. 2016, Ghadiri *et al*. 2016a, b, Shafiei *et al*. 2016, 2017). The best result of sports training is reducing the possibility of many severe and fatal diseases such as heart disease, osteoporosis, diabetes, obesity, high cholesterol, breast cancer, heart attack, and stroke and reducing arthritis (Ehyaei *et al*. 2017, Ghadiri *et al*. 2017a, b, Shivanian *et al*. 2017). Sports injuries occur during exercise (Chastin *et al*. 2021). Bone injury is one of the most common problems of athletes. Sports injuries are caused by heavy impact or prolonged and excessive stretching during sports activities that can affect bones, soft tissue (ligaments, muscles, and tendons), or both (Everard *et al*. 2021). Every year, many people suffer from sports injuries, half recover with home remedies or without special care, and the other half require medical attention. Bone fracture is one the types of sports injury (Johnson and Ivarsson 2017). Arm and leg bones are most prone to fracture. Fractures of the spine or skull during sports are very rare. The bones of the leg from the thigh to the bottom are very susceptible to fracture due to pressure and occur when the muscles become complicated or excessive contraction cause the bone to bend and break (Flanders and Bhat 1995). A leg fracture is accompanied by pain and swelling in the front, inside, and back of the leg, which becomes very painful during movement, and the pain is constantly worse (Cheng *et al*. 2000). This injury occurs due to high-impact movements or constant pounding of the foot on the ground in sports such as aerobics, endurance running, basketball, and volleyball (Briner and Kacmar 1997, Prado *et al*. 2022).

Rehabilitation is a set of coordinated medical, educational, professional, and social services and measures to rehabilitate the disabled person and improve his efficiency to the highest possible level to achieve an independent life in society (Christakou and Lavallee 2009). The relationship between rehabilitation and sports is

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precious. Rehabilitation helps people to achieve the highest level of performance, independence, participation, and quality of life, so the concept of sports and rehabilitation is similar (Ahern and Lohr 1997). Gutman first included physical activities and sports in rehabilitation services in 1991 (Hutzler and Sherrill 2007). In recent years, physical activities and sports have been used as words such as Adapted Physical Activity, Paralympics, Special Medicine Olympics, Health Promotion, and Rehabilitation. Nevertheless, Borms (2008) suggested adaptive physical activity as a service or profession in Rehabilitation, further linking the two sciences. After a bone fracture, a rehabilitation and physiotherapy program are considered for the patient (Ebrahimi *et al*. 2017, Ghadiri *et al*. 2017c, Shahabinejad *et al*. 2018, Shafiei *et al*. 2020). In some fractures, such as hip fractures, a rehabilitation program begins immediately, even while the individual is in the hospital. After surgery and fracture repair, getting out of bed and walking minimizes the possibility of bed sores and blood clots (Dhillon *et al*. 2017). Performing physical exercises in the hospital helps to strengthen and strengthen the injured area and surrounding muscles. Nevertheless, when an open bone fracture occurs, microbes can enter the bone from outside the body through the wound and grow there, causing bone infection (Greising *et al*. 2020). In some cases, bone repair requires orthopedic implants, Implants are instruments or tissues put inside or on the body's surface to replace lost tissue, which may cause bone infection and slow healing (Murray *et al*. 2016).

Zinc oxide, an inorganic compound depicted by the formula ZnO, is a white powder insoluble in water (Singh *et al*. 2011). Zinc oxide nanoparticles have various properties that make them used in various industries and applications on a large scale (Hou *et al*. 2021, Huang *et al*. 2021, Xu *et al*. 2021, Wang *et al*. 2022). Among these properties are high chemical stability, dielectric constant, high catalytic activity, low price, high adhesion and coating power, and the ability to recover, absorb and neutralize the sun's UV rays, and an essential property of this type of particle is that they are antibacterial and antiseptic (Kumar *et al*. 2013). It is stable with non-toxicity and human pathogen production. Furthermore, they are used in medicinal and health applications and various industries. Antibacterial or disinfectant properties of zinc oxide nanomaterials have made them a severe competitor for antibiotics (Sirelkhatim *et al*. 2015). The most serious weakness of antibiotics is the production of human pathogens, the result of which is the resistance and updating of bacteria against these disinfectants, and as a result, the need to use and make these drugs more potent daily (Happy *et al*. 2018). However, zinc oxide nanomaterials prevent the production of pathogens due to their anti-virus function, and there is no need to consume them in excess day by day (Pati *et al*. 2014). Antibacterial activity may cause the accumulation of these nanoparticles in the outer membrane or cytoplasm of bacterial cells and the release of zinc, which causes the separation of the bacterial cell membrane, membrane protein damage, and genomic instability, resulting in the death of the bacteria (Zhang *et al*. 2022a, b, Cao *et al*. 2023, Wang *et al*. 2023, Xu *et al*. 2023, Zhao *et al*. 2023). Also, these materials have high stability and do not harm the environment and humans due to their bio-compatibility (Menazea *et al*. 2021). Also, these materials are used in the production of dressings, bandages, burn ointments, and anti-inflammatory and anti-itching use (Liu *et al*. 2020, Wang *et al*. 2020, Zhou *et al*. 2020, Dai *et al*. 2021, Guo *et al*. 2021, Shao *et al*. 2021, Wu and Habibi 2021). There are different physical, chemical, and biological methods for the synthesis of zinc oxide, each of which is used depending on the conditions and purpose of synthesis, and the general methods are synthesis in the form of sol-gel, micro-emulsion, chemical mechanical process, direct solvent evaporation, hydrothermal, sedimentation, spark formation is used, which depends on factors such as surface chemistry, size distribution, particle morphology, and particle reaction in solution (Wojnarowicz *et al*. 2020). Terms of morphology and morphology, oxide nanoparticles have two crystal structures, in terms of morphology and morphology, oxide nanoparticles have two crystal structures, one is hexagonal, and the other is cubic, which is more common in the industry since the hexagonal structure is more stable than the cubic structure at ambient temperature (Ananth *et al*. 2017). The most serious weakness of antibiotics is the production of human pathogens, the result of which is the resistance and updating of bacteria against these disinfectants, and as a result, the need to use and strengthen these drugs daily. Nevertheless, zinc oxide nanomaterials prevent the production of pathogenic agents due to their antiviral function, and there is no need to consume them excessively daily. Antibacterial activity may cause the accumulation of these nanoparticles in the outer membrane or cytoplasm of bacterial cells and the release of zinc, which causes the separation of the bacterial cell membrane, membrane protein damage, and genomic instability, and as a result, the death of bacteria (Liaqat *et al*. 2022). Also, these materials are highly stable due to their bio-compatibility. They do not harm the environment and humans, these materials are used in the production of dressings, bandages, burn ointments, and anti-inflammatory and anti-itching (Saravanan *et al*. 2018).

Many living organisms such as bacteria, fungi, algae, plants, plant extracts, and their metabolites have been used to synthesize nanoparticles. Nevertheless, the identification of plant systems as potential natural nano factories has created a great interest in the biosynthesis of nanoparticles (Devatha and Thalla 2018). In the green synthesis of nanoparticles, natural compounds and biological agents in plant extracts such as enzymes, carbohydrates, and terpenoids replace harmful chemical compounds and solvents used in chemical methods. Therefore, the synthesis of nanoparticles using natural resources leads to a reduction in synthesis steps and the use of energy and chemical solvents that destroy the environment (Huston *et al*. 2021). Jones *et al*. (2008) studied the antimicrobial activity of zinc oxide nanoparticles. They described their results according to the hypothesis of the accumulation of nanoparticles inside the bacterial cell membrane and cytoplasm. They discovered that these nanoparticles show antimicrobial activity against Escherichia coli. They claimed that this activity results from an intracellular accumulation of

nanoparticles in the cells' bacterial membrane and cytoplasmic regions. Han *et al*. (2021) investigated zinc nanomaterials with different dimensions in rehabilitating patients with fractures and organ damage. They suggested that the unique Physico-chemical properties of ZnO nanomaterials can be used to prepare artificial bone, bone cement, and external fixation with good osteogenic performance and antibacterial performance and carry antibiotics to treat open fracture infection and bone defects. Liu and Wan (2021) discussed the value of using zinc oxide nanomaterials with different dimensions in the sports rehabilitation of basketball players injured due to limb fractures. Experimental data show that the different dimensions of ZnO nanomaterials have little effect on the success rate of the operation, but there are significant differences in the anti-infection aspect. Materials with zero dimensions performed well in the test. Compared with the one-dimensional group and two-dimensional group, the rehabilitation time and complications were effectively reduced. In this report, it is suggested that the use of ZnO nanomaterials with zero dimensions in fracture surgery is a treatment plan that can become common.

Therefore, in this research, in line with the purposes of green chemistry, green synthesis of zinc oxide nanoparticles using Hibiscus sabdariffa sepal extract as a biological source of synthesis, without using regenerating and stabilizing chemical agents, has been done for faster treatment of sports injuries and sports rehabilitation. So far, research on the synthesis of zinc oxide nanoparticles by sepals has not been done, and this synthesis is done for the first time in this work.

2. Materials

Table 1 shows the materials used for the green synthesis of zinc nanoparticles by tea extract for sports rehabilitation and treatment of sports injuries.

Table 1 List of materials and devices

Materials and devices
$Zn(CH_3CO_2)_2.2H_2O$
Ethanol
Peptone
Table salt
Yeast extract
Agar
Soxhlet
Escherichia coli bacteria
Bacteria Staphylococcus aureus
Oven
Centrifuge

3. Methods

3.1 Hibiscus sabdariffa sepal extraction

Extraction is extracting (active ingredient) by separation method based on solubility mechanism (aqueous, alcoholic, hydro alcoholic). 10 grams of Hibiscus sabdariffa sepal powder was put in the thimble of the Soxhlet apparatus, then 150 mL water was poured into the flask, then placed in a water bath under reflux conditions, and the extraction process lasted for 3 hours.

3.2 Green synthesis of zinc oxide nanoparticles

For the synthesis of nanoparticles, zinc acetate solution (dehydrate acetate zinc) was prepared with the chemical formula $Zn(CH_3CO_2)_2.2H_2O$ with a concentration of 0.07 M and pH equal to 11.5. Furthermore, added to the aqueous extract with a volume ratio of 5:5 (V/V). Then stirring was done for 1 hour at 60 ˚C. After completion, the reaction was allowed to sample for 24 hours to allow a white precipitate to collect at the bottom of the vessel. Then, the sediments were collected with the help of a centrifuge at 4000 rpm for 10 minutes. After being separated from the supernatant solution, they were washed twice with deionized water and ethanol to remove impurities. Then the collected sediments were dried in an oven at 60 ˚C. To create a powder form of the obtained sample, it was placed in an oven with a temperature of 400 ˚C for 2 hours.

3.3 Investigating the antibacterial properties

To study the antibacterial activity of Hibiscus sabdariffa sepal extract and zinc oxide nanoparticles, Methicillin-resistant Gram-positive pathogenic bacteria Staphylococcus aureus and Gram-negative Escherichia coli bacteria were used. In this method, fresh Escherichiacoli bacteria and Staphylococcus aureus bacteria were cultured with concentrations of 10^4 (Cfu/ml) in test tubes containing 10 ml of liquid culture medium. 0.04 grams of prepared nanoparticles were added to the tubes, and the resulting suspensions were incubated for 10 hours at 38°C. After incubating the suspension containing nanoparticles and the control sample to the size, they were diluted $10^{-2,}$ 10^{-3}, and 10^{-4} times and cultured on agar plates. Then the plates were incubated for 10 hours and 39 degrees Celsius. Finally, the number of colonies created in each case was counted and compared with the control sample. For each dilution, the experiment was performed three times.

4. The use of ZnO nanoparticles in sports rehabilitation

To use this nanoparticle to treat fractures without infection, 30 athletes who suffered bone fractures were randomly selected. 15 of them were selected with ZnO nanoparticles combined with sports rehabilitation as an experimental group, and 15 with traditional sports rehabilitation treatment as a control group were selected in each group. The experimental group was treated with nanoparticles at the fracture site and sports rehabilitation. In contrast, the control group was treated for 50 days by taking drugs prescribed by the doctor and traditional sports

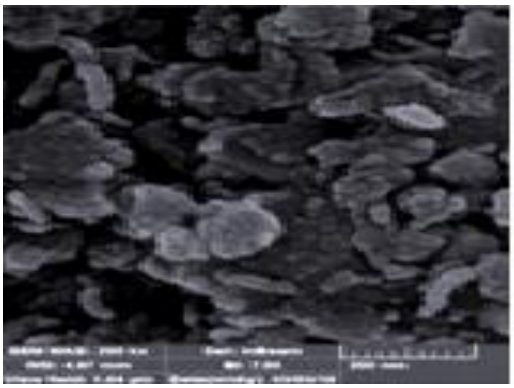

Fig. 1 SEM of ZnO nanoparticles

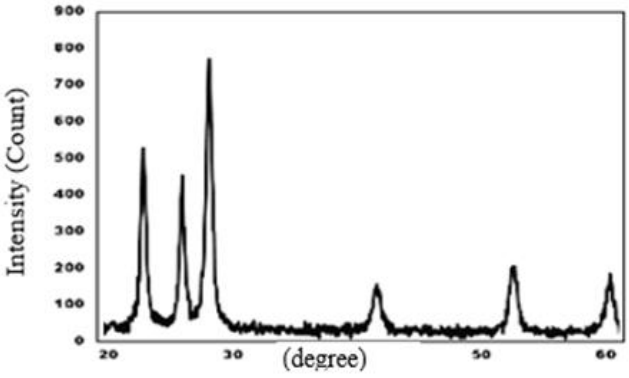

Fig. 2 XRD analysis of ZnO nanoparticles

recovery methods. Various microbes can cause osteomyelitis or bone infection, but the most common of them is the bacterium called Staphylococcus aureus, which is the cause of infection in 80% of cases. Bone infection or osteomyelitis can occur in any person, however, conditions such as fracture or bleeding inside the bone, the presence of a prosthesis or metal screw and plate inside the bone after previous surgery, and recent surgery on the bone make it more likely to occur. After 50 days, the patients in two experimental and control groups were examined by a doctor. The results showed that the patients in the zinc oxide nanoparticle treatment group had shorter rehabilitation times and infection-free fracture sites than the control group. The incidence of side effects in the experimental group was almost zero.

5. Results

5.1 Examining the morphology of nanoparticles

A field emission scanning electron microscope was used to evaluate the size and morphology of zinc oxide nanoparticles. The resulting microscopic images (Fig. 1) showed that more Synthesized nanoparticles have a spherical shape. Also, the analysis results show the accumulation of nanoparticles caused by applying the drying process in the oven. According to the microscopic results, the size distribution pattern showed that the size of ZnO nanoparticles is less than 40 nm.

5.2 X-ray powder diffraction

XRD analysis or X-Ray Diffraction is a fast-analytical method primarily used to identify the phase of a crystalline material and can deliver details about it. The function of the XRD device is that the X-ray is irradiated to the crystal at different angles (θ). As a result of this radiation and the impact of the ray on the atoms, the ray is reflected or diffracted. Ray reflection in XRD follows the principle of Rayleigh scattering. The radiated and diffracted beam frequency is the same, and a photon is not absorbed when it hits an atom. In XRD, there is also fluorescence radiation, and part of the photons are absorbed by atoms whose reflected optical filters remove rays. XRD is a low-cost and widely used method due to its simple physical principles. The information obtained from this method, which includes the angle of the maximum intensity of the scattered rays, the intensity of the scattered rays at each angle, and the width of each maximum, can determine a wide range of properties and quantities of crystals, and this causes countless applications of XRD. The XRD pattern of nanoparticles synthesized by a green method is shown in Fig. 2. In this figure, all the recognizable peaks are related to the zinc oxide structure. The significant peaks' width can indicate the particles' nanometer size. The size of the particles obtained by the XRD was about 39 nm, which was consistent with the result of SEM.

5.3 Antibacterial activity

The antibacterial activity of Hibiscus sabdariffa sepal extract and zinc oxide nanoparticles synthesized with its help was investigated on methicillin-resistant Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli bacteria. Staphylococcus aureus is the most dangerous common staphylococcal bacteria. These gram-positive, spherical bacteria often reason skin infections but can also reason pneumonia, heart valve infections, and bone infections. Many strains show resistance to antibiotic effects. If the carriers take antibiotics, the antibiotics will kill the strains that are not resistant, and the resistant strains will mostly remain. These bacteria may multiply, if an infection develops, it is more challenging to treat. Escherichia coli, or E.Coli is a gram-negative intestinal bacillus in the Enterobacteriaceae family, which is more isolated than other species and causes various infections.

The colony unit counting method was used against bacteria to investigate the antimicrobial activity of nanoparticles. The composition of the culture medium used includes 8 gr/lit of Peptone, 8 gr/lit of table salt, 5 gr/lit of yeast extract, and 15 gr/lit of agar. In order to calculate, the number of colonies formed on each container containing the nutrient culture medium was counted. The images of bacteria culture medium containers in the presence of nanoparticles and their absence are shown in Fig. 3. Fig. 3.a show the culture medium of Escherichia coli in the presence of ZnO nanoparticles. Fig. 3.b shows the bacteria Escherichia coli in the absence of nanoparticles. Fig. 3.a' shows the culture medium of Staphylococcus aureus in the presence of ZnO nanoparticles. Fig. 3.b' shows the culture

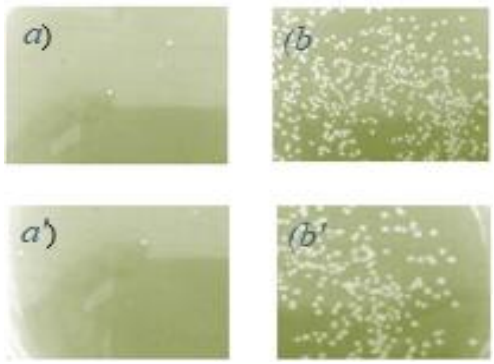

Fig. 3 Bacteria culture medium, a: Escherichia coli in the presence of ZnO nanoparticles, b: bacteria Escherichia coli in the absence of nanoparticles, a': Staphylococcus aureus in the presence of ZnO nanoparticles, b': Staphylococcus aureus in the absence of nanoparticles.

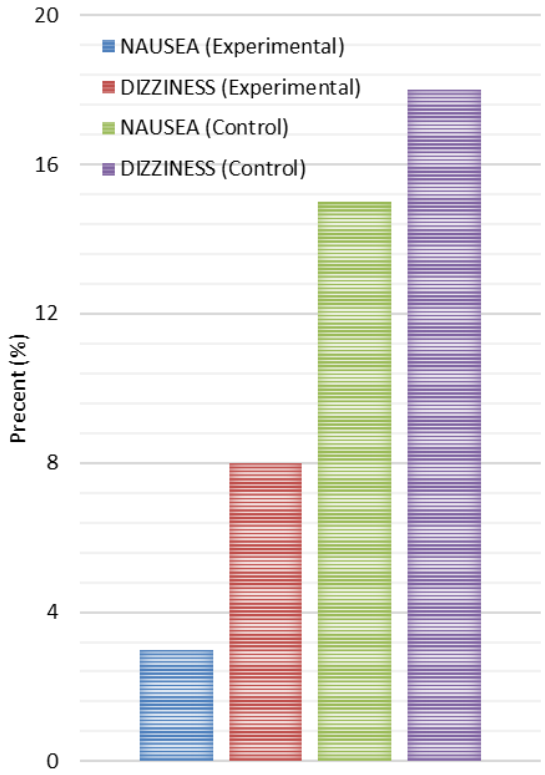

Fig. 4 Comparison of analgesic adverse reactions between the two groups

medium of the bacteria Staphylococcus aureus in the absence of nanoparticles. As seen in the figure, two bacteria have grown very weakly in the culture media containing nanoparticle ZnO, and their growth has stopped and disappeared. The antimicrobial activity of nanoparticles based on the relationship (B-A/B)100=R was calculated. Where R is the reduction percentage, A is the number of bacteria counted in the sample (Cfu/ml), and B is the number of colonies in the control sample (Cfu/ml). Based on the calculations, the average antimicrobial performance of zinc oxide nanoparticles is more than 98%. Here, zinc oxide nanoparticles have directly destroyed the bacterial membrane and caused its destruction.

5.4 Unfavorable effects

Unfavorable Effects in the Experimental Group Shown in Fig. 4, the incidence of Unfavorable responses in the experimental group was 11%, among which NAUSEA accounted for 3%, and DIZZINESS and headache accounted for 8%. The incidence of adverse reactions in the control group was 33%, including 15% NAUSEA and 18% DIZZINESS and headache. The incidence of Unfavorable reactions in the experimental group was significantly lower than in the control group ($P < 0:05$).

6. Discussion

Today, the role of sports and sports sciences in providing and improving the health and lifestyle of people in society is prominent. Injuries are inevitable in sports. The goals of sports rehabilitation are to treat and restore the function of the injured athlete's body as quickly as possible. This means that rehabilitation is done simultaneously as a treatment to minimize the effects of the injury. Influential factors in sports rehabilitation include injury severity, treatment stage, medical or surgical treatment, and muscle strength of the injured limb. Resuming sports activities after injury and sports rehabilitation and physical therapy will cause fear and anxiety in athletes by re-intensifying the symptoms of a disease. However, starting sports activities as soon as possible accelerates the recovery process and the patient. Physical activity will improve health. Increasing physical activity and reducing inactivity will prevent the occurrence of chronic diseases. The patient cannot do physical activity when pain or injuries limit mobility. Limiting the range of body movements will lead to muscle and bone weakness and will disrupt the fitness of the cardiovascular system. In the early stages of rehabilitation, symptoms of inflammation, such as swelling and pain, are considered a factor in determining the level of sports activity. Even after returning to the sports field, doing some special rehabilitation exercises is necessary so that the patient does not get injured again. Nano is one of the emerging branches of technology that researchers, technologists, and entrepreneurs have welcomed in recent years due to its wide applications in various products and industries. The growing trend of using nanomaterials and products in various fields, including agriculture, medicine, and industry, has made nanotechnology one of the pioneers of technology. One of the exciting applications of nanotechnology is sports, and the published news of the development and commercialization of products based on nanotechnology in this industry shows its potential. It has been proven that nanotechnology can have useful applications in sports and cover many sports fields, including sports textiles and clothing, sports equipment, and even medical equipment for athletes. Nanotechnology has provided new opportunities for producing innovative materials that not only meet the needs of athletes but also help them to be at the highest level of safety and comfort and provide their best performance in competitions. The entry, growth, and development of microbes or parasites in a wound or organ of a living organism is called an infection. The organism that the microbe uses for growth is called the

host. If an infection occurs, the host's body will give an inflammatory response to it, and this response will cause symptoms of the disease. Pathogens include parasites, viruses, bacteria, and fungi. Infection can occur after a broken bone surgery in athletes, and the healing process of the injured limb becomes more protracted and more painful. Using ZnO nanoparticles synthesized greenly, it is possible to help the injured member recover quickly and prevent the accumulation of microbial agents that cause infection. The athlete can return to the routine by performing appropriate exercises and sports.

7. Conclusions

In conclusion, the ongoing development of antibiotic drugs and disease treatments has led to an increase in their availability. However, the widespread production and misuse of antibiotics have resulted in the emergence of drug-resistant infectious diseases. The uncontrolled growth of various microorganisms, which normally coexist in harmony with the human environment, can give rise to severe complications. Within cells, ZnO nanoparticles exhibit biological effects. The primary mode of action of these nanoparticles against bacteria involves damaging proteins and destroying cell walls. As the concentration of nanoparticles increases, their antibacterial activity becomes more pronounced. The utilization of plant materials for synthesizing nanoparticles offers several advantages, including the absorption of metal ions by plants and a better understanding of the nanoparticle formation process in plants. This article has explored the application of nanomaterials in the context of sports injuries and their therapeutic effects on sports rehabilitation. The use of ZnO nanoparticles brings about various changes in the damaged bone activities associated with sports-related injuries, enabling a more accurate understanding of the disease process and investigation into the impact of nanoparticles on treatment. Consequently, this promotes medical research, stimulates innovative problem-solving approaches, and establishes a solid theoretical foundation for addressing sports injuries and related conditions like post-surgical infections following bone fractures. By comparing and analyzing specific experimental outcomes, it becomes possible to identify the optimal equilibrium between the body's response and the effectiveness of nanoparticles, thereby providing valuable technical insights for future experiments and identifying similarities and differences in research directions. Furthermore, through comparative advantage analysis and the assimilation of advanced experiences, this research contributes theoretical grounds for the field of medicine, offers recommendations for advancing technological developments in future medical practices, and enhances expertise and precision.

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