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RESEARCH PAPER

The Efficacy of Manganese Oxide (Mn_2O_3) Nanoparticles and Tellurium Oxide (TeO_2) Nanorods Against Leishmania Lesions in Female Albino Rats

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ABSTRACT

Leishmaniasis is a group of diseases caused by infection with Leishmania parasites. The lesions that develop as a result of leishmaniasis can vary depending on the species of the parasite and the type of leishmaniasis. Cutaneous leishmaniasis is the most common form of the disease and it results in skin sores or ulcers. Materials with manganese oxide (Mn_2O_3) nanoparticles and tellurium oxide (TeO_2) nanorods have been shown to have antibacterial, antifungal, and antiparasitic effects. The purpose of this study was to ascertain how Mn_2O_3 and TeO_2 nanoparticles affected Leishmania major-caused wound healing in rats. The albino rats were separated into four groups of five once a lesion appeared on their tails. In the two treatment groups, Mn_2O_3 and TeO_2 nanoparticles were injected every day, once a day, intra-wound in three places, and in the meglumine antimoniate group, the drug was injected intramuscularly for five weeks. The albino rats in the negative control group did not receive any medication. The size of the wounds in the group treated with Mn_2O_3 nanoparticles did not differ significantly from the control group that did not receive treatment, however the diameter of the wounds in the group treated with TeO_2 nanorods did change significantly from the control group that did not receive treatment. It was, however, larger than the group that received meglumine antimoniate treatment. TeO_2 nanorods, as opposed to Mn_2O_3 nanoparticles, had an in vivo anti-Leishmanial potential.

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INTRODUCTION

Leishmaniasis is a parasitic disease caused by various species of Leishmania, which are single-celled protozoan parasites [1]. The disease is transmitted to humans and animals through the bite of infected female sand flies. There are three main forms of leishmaniasis: cutaneous leishmaniasis (CL), which causes skin sores;

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mucocutaneous leishmaniasis (ML), which affects the nose and mouth; and visceral leishmaniasis (VL), also known as kala-azar which affects the internal organs such as the liver, spleen, and bone marrow [2,3]. The disease is endemic in many tropical and subtropical regions, particularly in the Middle East, Africa, and Latin America, and can be a serious public health problem in these areas. Leishmaniasis can be difficult to diagnose and treat, and in some cases, it can be fatal if left untreated. Treatment typically involves medication, and in severe cases, hospitalization may be necessary [4–6].

Leishmania major is an obligate intracellular protozoan parasite that primarily infects mononuclear phagocytes, such as macrophages and dendritic cells, in the skin [7,8]. Following the sand fly bite, Leishmania major promastigotes are phagocytosed by mononuclear phagocytes and then transformed into amastigotes within the phagolysosome of the host cell [9–11]. The amastigotes then multiply within the host cell and can evade the immune system, leading to the development of the characteristic nodules or ulcers [12]. Sand flies are known to bite a variety of hosts, including humans, domestic animals such as dogs and cats, livestock such as cattle and sheep, as well as wild animals such as rodents and small mammals [5]. The female sand fly requires a blood meal to develop eggs, and in the process of obtaining blood, she can transmit various pathogens to the host, including the protozoan parasites that cause leishmaniasis, as well as viruses and bacteria [9,11].

It is estimated that there are approximately one million new cases of leishmaniasis each year worldwide [13]. VL, the most severe form of the disease, is estimated to cause approximately 20,000 to 30,000 deaths per year, primarily in India, Bangladesh, Sudan, South Sudan, Ethiopia, and Brazil [14,15]. Egypt is considered to be one of the countries with a high incidence of CL, particularly in the rural areas of the country [16,17]. A study estimated that there were approximately 20,000 cases of CL in Egypt each year, with a prevalence rate of around 23 cases per 100,000 population [18]. The treatment of Leishmaniasis depends on the severity and type of the disease, as well as the geographic region in which the infection occurs. There are several drugs used to treat leishmaniasis, including pentavalent antimonials, amphotericin B, miltefosine, and paromomycin [19,20]. Meglumine antimoniate is a pentavalent antimonial drug that is used to treat leishmaniasis caused by certain species of the Leishmania parasite especially in Egypt. It is administered through injection and is commonly used for the treatment of CL, as well as some forms of VL. Meglumine antimoniate works by inhibiting enzymes within the Leishmania parasite, leading to their death. While Meglumine antimoniate is an effective treatment for leishmaniasis, its use is limited by its toxicity and side effects, which can include nausea, vomiting, abdominal pain, muscle and joint pain, and cardiac toxicity [21].

In light of the aforementioned issues, researchers are considering using certain novel compounds, such as a solution of various nanoparticles. Nanoparticles are microscopic particles that typically range in size from 1 to 100 nanometers. They can be made of various materials, including metals, metal oxides, polymers, and biological substances, and are commonly used in a wide range of applications, including medicine, electronics, and environmental remediation [22,23]. One of the unique properties of nanoparticles is their large surface area to volume ratio, which can make them more reactive than larger particles of the same material. This property can be exploited in a variety of applications, such as in the development of more effective drug delivery systems or in the catalysis of chemical reactions [22].

Nanoparticles have shown promise as a potential tool for fighting parasites in medicine. Some studies have explored the use of nanoparticles for drug delivery in the treatment of parasitic infections, including leishmaniasis [24]. Other studies have explored the use of nanoparticles themselves as antiparasitic agents. For example, nanoparticles made of metals such as silver, gold, and copper have been shown to have potent antiparasitic activity against a range of parasites, including Leishmania [25]. Even though there has been credible research on the impact of tellurium oxide (TeO_2) nanorods on leishmaniasis, the conclusions drawn from them have occasionally conflicted. It's possible that the kind of parasite present affects how well the medication works. On the other hand, the impact of manganese oxide (Mn_2O_3) nanoparticles on leishmaniasis has received very little research. This investigation aimed to assess the impact of Mn_2O_3 nanoparticles and TeO_2 nanorods on the healing of lesions brought on by the Egyptian strain of

Leishmania major in albino rats.

MATERIALS AND METHODS

Two modified NNN (Novy-MacNeal-Nicolle) and RPMI 1640 (Roswell Park Memorial Institute 1640) mediums were employed in this experimental study [26]. The parasite was cultured in RPMI1640 media after first being grown in modified NNN medium. Dai-ichi Pure Chemicals (Tokyo) provided RPMI1640 medium for Leishmania parasite cultivation. Fetal calf serum (10–12%), 80 g/ml of streptomycin, and 80 units/ml of penicillin were supplemented into the medium to help stop the growth of bacteria [27]. The flasks were then placed in the 25°C incubator, where they were checked daily using an inverted microscope. A new

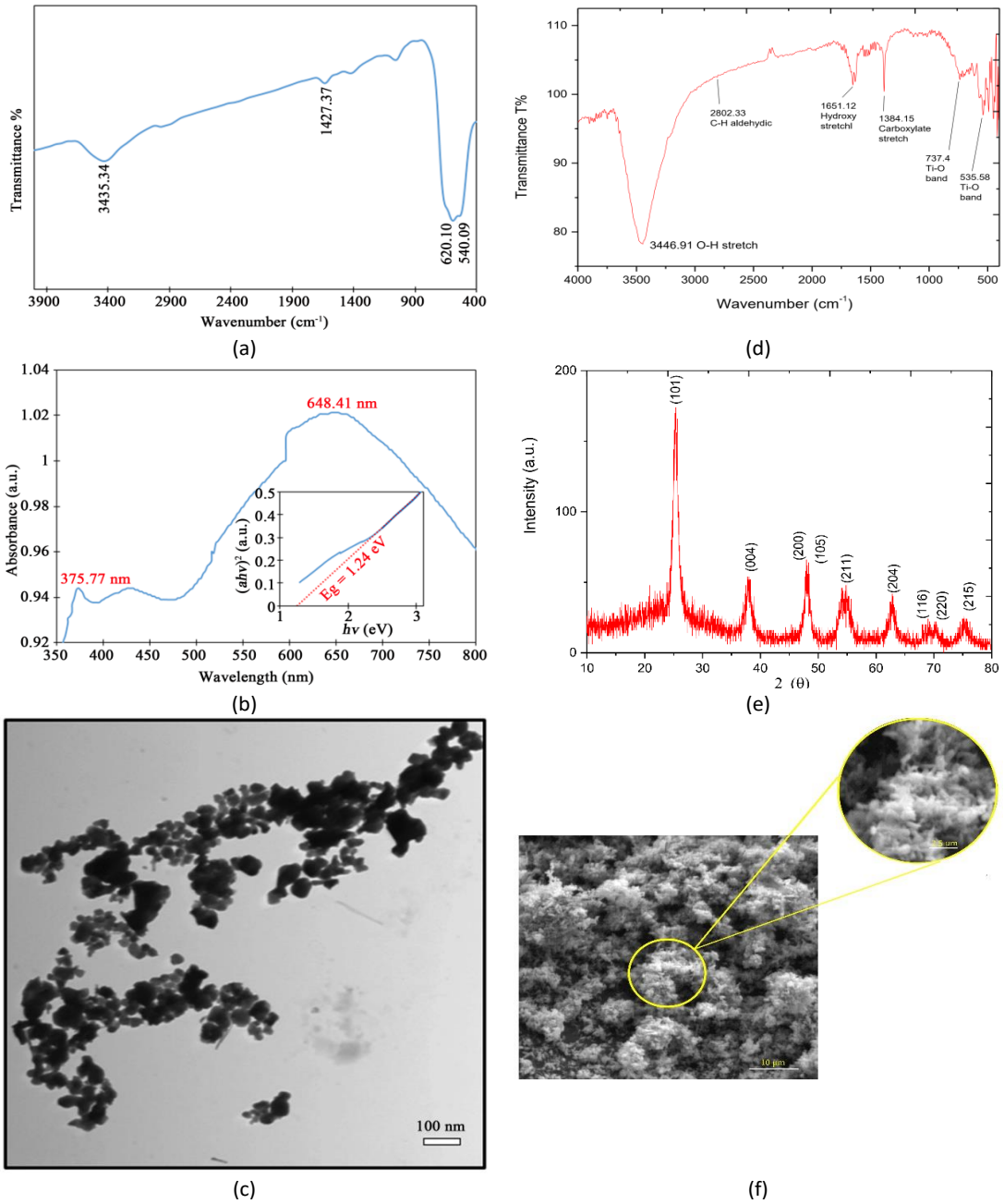

Fig. 1. Mn_2O_3 nanoparticles: (a) FTIR spectroscopy, (b) UV-spectroscopy, and (c) TEM image; and TeO_2 nanorods: (d) FTIR spectroscopy, (e) XRD pattern, and (f) SEM image.

culture medium is added to the promastigotes if the medium becomes yellow and they reach the stationary phase. This task is repeated until the necessary number of parasites is reached. Hemocytometers were used to count the number of parasites.

In this study, to evaluate nanoparticles in vivo, nanoparticle injection with a concentration of 135 mg/kg was used as an intra-wound injection at three points once a day for four weeks. Female albino rats (Wistar strain weighing 150–200 g) older than five weeks acquired from animal farm of the Egyptian Organization for Biological Products and Vaccines (VACSERA Holding Company), Cairo, Egypt were the subjects of this study. An insulin syringe was used to administer 0.15 ml of a solution containing 1.5×10^6 promastigotes of Leishmania major in the stationary phase subcutaneously into the base of the rats' tails. It should be noted that following cultivation in the modified NNN environment, the number of promastigotes was counted daily in order to validate the parasite's stationary phase. As the parasite enters the stationary phase, its growth slows. A little firm nodule at the injection site started to form after two weeks of parasite injection; after another two weeks, the nodule turned into a wound. To confirm the presence of Leishmania parasites in the wound, the direct slide technique was used for sampling and examination under the microscope.

The rats were marked and put in different cages using the picric acid staining technique to distinguish them from one another. Rats were divided into four groups (infected control without treatment, infected group treated with Mn_2O_3 nanoparticles, infected group treated with TeO_2 nanorods, and infected control group treated with meglumine antimoniate) and five rats were placed in each group. The wounds were fully visible after 14 days. Mn_2O_3 nanoparticles and TeO_2 nanorods were injected into the wound at three points once a day for four weeks. The fourth group of rats received an intramuscular injection of meglumine antimoniate (25 mg/kg) once a day for four weeks. For a period of five weeks, measurements and records were made of the rats' weight and the size of the wound. Transmission electron microscopy (TEM) image, scanning electron microscope (SEM) image, X-ray diffraction (XRD) pattern, UV-visible optical spectroscopy, and Fourier-transform infrared spectroscopy (FTIR) of the Mn_2O_3 nanoparticles and TeO_2 nanorods employed in this investigation are all displayed in Fig. 1.

One-way ANOVA was used to compare the mean of the researched variables across various groups. Also, t-test was used to detect significant differences between different groups. To check for the assumption of normality of the investigated variables, the one-sample Kolmogorov-Smirnov test was employed. The data were analyzed using SPSS version 16 software, and a significance level of 0.05 was taken into account.

RESULTS AND DISCUSSION

The results of the average wound diameter of the control and experimental groups during five weeks are shown in Fig. 2. The wound did not completely heal in any of the groups. The meglumine antimoniate group had the least wound diameter. The difference between the mean size of the wound diameter of this group and other groups was significant ($p<0.05$). The mean wound diameter between the group treated with Mn_2O_3 nanoparticles and the control group with no treatment did not differ significantly ($p>0.05$). However, compared to the groups receiving meglumine antimoniate and TeO_2 nanorods treatments, this group's wounds had a considerably greater diameter ($p<0.05$). In comparison to the untreated control group, the mean diameter of the wounds in the TeO_2 nanorods group was significantly different ($p<0.05$). Overall, the findings demonstrated that using Mn_2O_3 nanoparticles and TeO_2 nanorods in female albino rats did not result in a full healing of the wound caused by Leishmania major.

Throughout the course of the treatment (35 days), rats had their weight assessed five times. The mean value and standard deviation of the weight of rats in different groups during the study period are shown in Fig. 3. In comparison to the untreated control group, the weight of the rats in the Mn_2O_3 nanoparticles and TeO_2 nanorods treatment groups was significantly different ($p<0.05$).

The use of nanotechnology has expanded in various fields in recent years, including medical sciences. Nanoparticles have been studied extensively for their potential use in treating various diseases, including infections caused by microorganisms such as bacteria, viruses, and fungi [28]. Mn_2O_3 nanoparticles and TeO_2 nanorods are examples of nanoparticles that have shown antimicrobial and antifungal effects

in experimental studies. For instance, research has demonstrated that Mn_2O_3 nanoparticles can inhibit the growth of bacterial strains such as Escherichia coli and Staphylococcus aureus, while TeO_2 nanorods have been shown to have antifungal activity against Candida albicans [29–32]. Narayanan et al. [33] examined the effects of various TeO_2 nanorods concentrations on the Leishmania major parasite in vitro and in vivo. The results of this study showed that different concentrations of TeO_2 nanorods in comparison with the control group cause a decrease in amastigotes, but this decrease did not have a significant difference with the control group. Moreover, no significant difference in the average size of wounds was seen between TeO_2 nanorods concentrations. On the contrary, in the present study, the consumption of TeO_2 nanorods caused

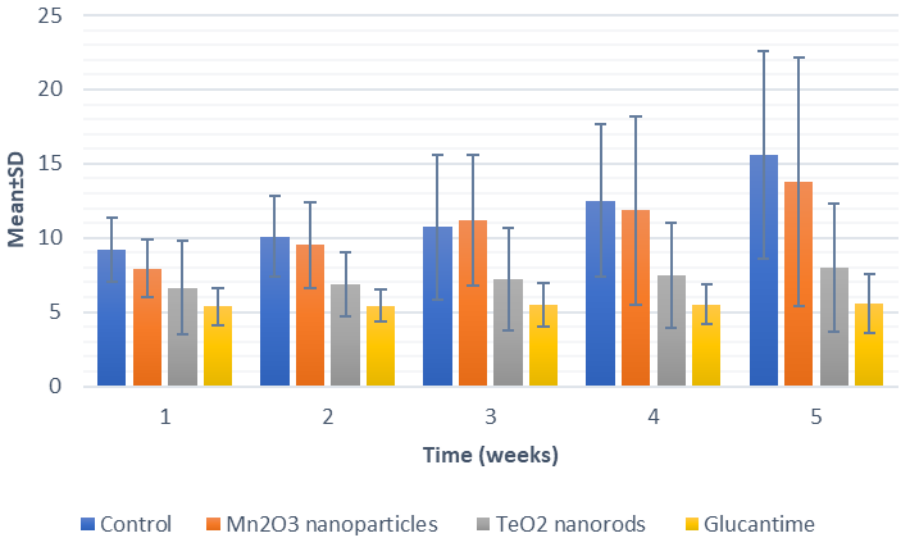

Fig. 2. The mean ± standard deviation of the wound size in the rats under study in the treated and control groups (mm).

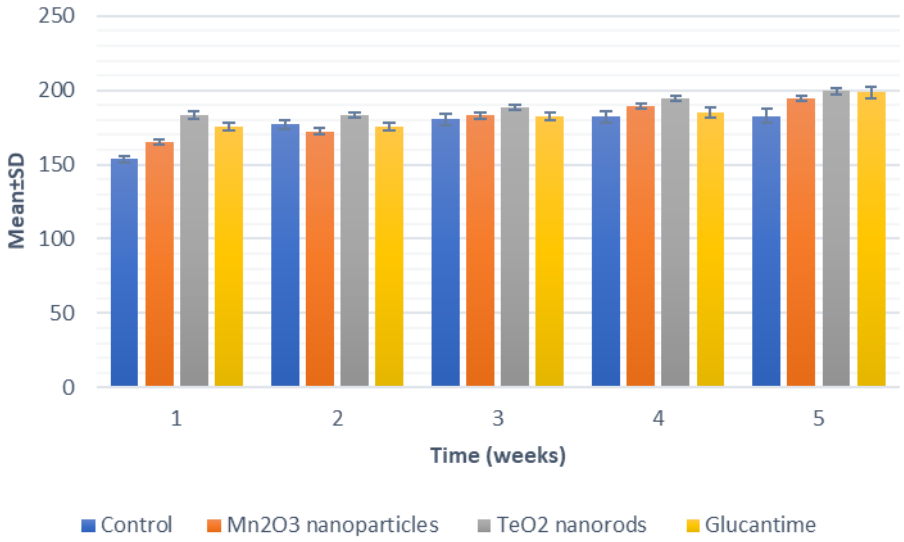

Fig. 3. The mean ± standard deviation of the weight of the rats under study in the treated and control groups (g).

a significant decrease in the average growth of the wound diameter, so that after five weeks of follow-up, the size of the wound in the group treated with TeO_2 nanorods was about half of the size of the negative control group (without receiving any treatment). Considering that a single strain was used in both of these studies, the role of the strain in the effect of TeO_2 nanorods nanoparticles on Leishmania major is completely ruled out. Regarding the mechanism of TeO_2 nanorods effect, it is known that TeO_2 nanorods produce reactive oxygen species [34] and Leishmania parasite is very sensitive to it. In the research conducted to determine the effect of topical treatment of skin wounds with TeO_2 nanorods, it was shown that TeO_2 nanorods probably do not cause toxic effects on hemoglobin and liver function in laboratory white mice [35]. Regarding the use of Mn_2O_3 nanoparticles against CL, there have been limited studies that evaluated the effects of different concentrations of biogenic Mn_2O_3 produced by Bacillus species MSH-1 on Leishmania major in vivo [31,32,36–38]. The results showed that the wounds of the mice that received Mn_2O_3 nanoparticles at a dose of 5 and 10 mg/kg for 14 days before the parasite was injected intraperitoneally into the mice were smaller than the others. The wound of the mice that received Mn_2O_3 nanoparticles at a dose of 5 and 10 mg/kg for 14 days after the parasite was injected intraperitoneally was completely removed. However, in the present study, which injected Mn_2O_3 nanoparticles into the wound at three points for four weeks, the results showed that the diameter of the wound in the group receiving Mn_2O_3 nanoparticles was not much different from the control group without treatment. The difference between these two studies may be due to the different type of Mn_2O_3 nanoparticles used in terms of source and size or its injection method.

CONCLUSION

The results showed that the use of Mn_2O_3 nanoparticles does not have much effect on the healing process or reducing the size of the wound caused by Leishmania major. On the other hand, TeO_2 nanorods in rats, although it limits the wound, but it does not cause the complete healing of leishmania wound.

CONFLICT OF INTEREST

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

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