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The protective role of nanoextract *Origanum. majorana* leaves and histological changes in the lungs of albino rats treated with Leishmania donovani

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Abstract

The impacts of zinc oxide nano-extract from *O. majorana* leaves on the histological structure of the lung of albino rats were investigated in the current study. There were fourty- two rats that are rats are divided into six groups of seven animals. The first group (negative control) received 0.3 ml of normal saline subcutaneously. The second group (positive control group) received *L. donovani* parasite injections. The third group treatment with nano extract of zinc oxide (10 mg/kg) from *O. majorana* leaves was given subcutaneously. The fourth was treat with nano extract of zinc oxide (15 mg/kg) from *O. majorana* leaves, the fifth, and sixth groups got nano-extractors (10 and 15 mg/kg) and were infected with *L.donovani*. The animal was subsequently given 0.3 ml of nano-extract three times a week for a month. Moreover, the findings revealed that there are several changes in their lung structure; inflammatory cell infiltration into the alveolar gaps, eosinophilic inflammation, regenerative proliferation of airway epithelial cells, and thickening of the bronchial muscle wall were frequently seen. After exposure to low levels of ZnO NPs., lymphocytes and eosinophils grew somewhat. Mild increases in lymphocytes and eosinophils were seen after exposure to low doses of ZnO NPs in *L.donovani*-infected tissue. The histological changes on the lung treated with Nano-extract (zinc oxide) of the *O. majorana* leaves at different concentrations were employed for
treating the histological shifts and reducing the damage caused by the parasite. Thus, the effectiveness of the nano-extract is effective in reducing the influences and resisting the parasite. Histological changes in the lung in groups treated with the nano extract showed resistance to the parasite in terms of its lack of effect on causing histological

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changes compared to the positive control group. The concentration of the crude essential oil, which was (15mg/ml), gave the highest percentage of growth inhibition for the parasite lung tissue and showed few fibrosis around the alveolar sacs in the group treated. In contrast, the second concentration of the essential oil, which was (10mg/ml), showed a lower percentage of inhibition and cellular killing of the parasite's visceral cells.

Aim of study: There is a need for novel therapeutic techniques. Recent breakthroughs in nanosystems have investigated passive and active targeting ways to increase medication concentrations while reducing undesirable toxicity and side effects to healthy tissues. The utilization of nanoparticles for targeted drug delivery has the potential to reduce medication dosage and address difficulties linked to conventional drug treatment, including rapid clearance, insolubility in aqueous environments, and a lack of selectivity that leads to non-specific cellular toxicity.

Keywords: *Origanum. Majorana, Leishmania donovani, lungs, albino rats zinc oxide nano-extract,*

Introduction

The manipulation of matter on a molecular scale is often characterized as nanotechnology. This relates to the creation of structures and even devices known as nanomaterials with dimensions ranging from 1 to 100 nanometers [3]. "Nano" and "technology" are the two components that comprise the term nanotechnology. The suffix "technology" pertains to the development and application of technical methods, whereas the prefix "nano" originates from the Greek word "nanos," which means miniature [2]. Plant extracts provide a viable substitute for physical and chemical approaches in the manufacture of zinc oxide nanoparticles (ZnO NPs) since they may be made from a variety of plant components, including leaves, stems, seeds, roots, and fruits, without releasing harmful chemicals into the environment. The utilization of natural extracts from plant parts is an ecologically beneficial and low-cost approach that yields a high-purity, impurity-free product [4]. Plants are the most popular source for NP synthesis due to the fact that they produce stable NPs in an extensive variety of shapes and sizes. Plant-released phytochemicals include polysaccharides, polyphenolic compounds, vitamins, amino acids, alkaloids, and terpenoids eliminate metal ions and oxides [20].Leishmaniasis is an overlooked tropical disease that poses a medical challenge in 98 countries, with an annual mortality rate of approximately 50,000 people (GBD 2013). More than three hundred and fifty million additional people are at risk, with 1.5 to 2 million new cases of leishmaniasis reported

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annually, of which 500,000 cases are visceral leishmaniasis and 1 to 1.5 mill on cases are cutaneous leishmaniasis [11]. The most important signs to determine the infection are weight loss, enlargement of the liver and spleen, and pancytopenia, all of which are symptoms that are expected to appear in a routine blood test. Without treatment, [1].

The lungs consist of 300 million alveoli, which are sacs shaped like cups and enclosed by a network of capillaries. Oxygen from each alveolus bonds to hemoglobin when red blood cells pass through the capillaries. The diaphragm and rib muscles flex during inhalation, expanding the chest cavity's capacity. The alveoli are forced to expand and fill as a result of the rise in air pressure, which lowers the air pressure in the chest cavity. This procedure passively obtains air from the environment for the lungs. The diaphragm and ribs relax, the chest cavity shrinks, and internal air pressure rises as we exhale. Compressed air forces alveoli to shut, releasing air. The diaphragm and ribcage receive neurological signals from nerve fibers that enter the chest cavity and control respiration. Blood carbon dioxide affects nerve impulses and breathing rate. Increased carbon dioxide causes more nerve impulses [4].

Methodology

The nano extract was prepared from *O. majorana* leaf powder. Initially, the ethanolic extract of seeds extracted from the leaves of O. majorana was prepared, with some modifications, according to the method [9]. Some practical modifications [6] have been made to produce nanoparticles (zinc oxide) from the ethanolic leaf extract of *O. majorana*. The research used 42 adult male white rats of the Rattus ratus species, weighing between 250 and 300 grams and aged between 7 and 10 weeks. Plastic animal husbandry boxes with iron clips on top were used to hold water bottles. Cages are always cleaned. The animals are kept in acceptable laboratory conditions with 12 hours of light and 12 hours of darkness at a temperature of 20 to 25 degrees Celsius. The animals were placed in an acclimatization period of 14 days. During this period, they were given water and food on a regular basis [7].

42 albino mice were used in the experiment. The animals were divided into six groups, with seven individuals in each group. The first group (negative control group) was treated with a subcutaneous injection with normal saline, the second group (positive control group) was treated with a subcutaneous injection the parasite *L. donovani*. The third group of animals was treated with (10 mg/kg) of nano extract subcutaneously. The fourth group was

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treated with a nano extract (15 mg/kg), the fifth group was treated with a nano extract (10 mg/kg), and L. donovani was also treated in the sixth group, which received a nano extract at a rate where the animal was injected with the nano extract three times a week for one month. After the end of the first experiment, each animal used in the experiment was dissected. The purpose of the autopsy was to evaluate the effect of different doses (10 and 15 mg/kg) of nano zinc oxide prepared from O. majorana leaf extract on lung histological structure. Anesthesia was performed by injecting a mixture of 3 ml of xylazine and 1 ml of ketamine [26] .

L. donovani parasites were grown and activated in Novy-MacNeal-Nicolle (NNN) media. Then it was placed in (RPMI-1640) medium with 1% (penicillin and streptomycin) and 10% FBS serum. Which was sterilized, and after 72 hours, it was placed in an incubator at 26 degrees Celsius, which is the appropriate temperature for the development stage of the flagellate parasite [27]. To confirm the growth of the parasite, the culture medium was previously examined by taking a slide equipped with a 40x lens and examining it under an optical microscope. When the appearance of the flagellar stage of the parasite was confirmed, 0.5 ml of the isolate was transferred [8]. After collecting the promastigote stage of the parasite, it was placed in a centrifuge at 1500 rpm for 10 minutes. The supernatant was collected using Pasteur pipettes. The concentration was measured using a hemocytometer to 1.2106 parasite promastigote cells per ml, depending on the number of parasites per ml. The parasites present in each of the 16 small squares were counted. N × 10 × 1000 × 20 represents the total number of cells per ml (N = number of cells counted). 10 represents the number of cells in lmm3. 1000 = number of cells per ml. Dilution factor (20) [13].

Results

The results showed the presence of histological changes in the lungs in the groups treated with the parasite-resistant nano extract, as no histological changes were found compared to the control group. Treatment with the nano extract (15 mg/ml) showed the highest rate of growth inhibition of the parasite. While the second concentration (10 mg/ml) showed a lower percentage of inhibiting and killing the parasite. As in Figure 1, the results of the microscopic examination of the lungs of the control group showed normal structure and no changes in the lung tissue. Figure 2, When animals were injected with the L. donovani parasite as a positive group, histological changes, congestion, lymphocyte infiltration, and blood cell aggregation were observed. In Figure 3, after receiving 10 mg/kg of the nano-

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extract of O. majorana leaves by injection, Figure 4. That when the extract was given O. majorana leaf nano extract at a dose of 15 mg/kg Fig. 5 Inflammatory cells invaded the lung treated with a dose of 10 mg/kg of majorana leaf nano extract with L. donovani. As shown in Figure 6, the lung that received a 15 mg/kg injection of L. donovani, a nano-extract derived from O. majorana leaves, showed the presence of atypical cells, minor hemorrhages, and inflammatory cells..

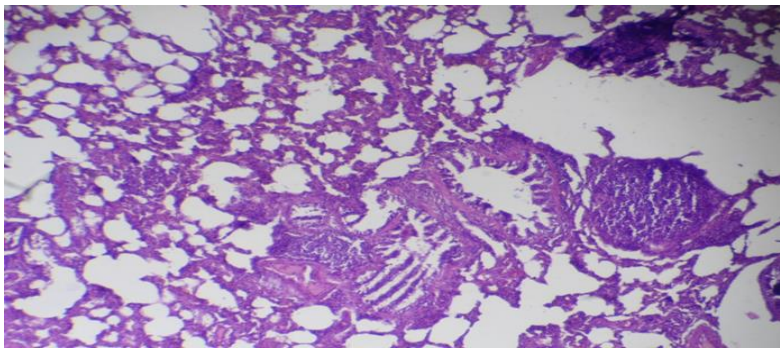

Fig. 1: Cross-section of lung tissue showing the normal alveolar sac structure of the negative control group (40x) (H&E).

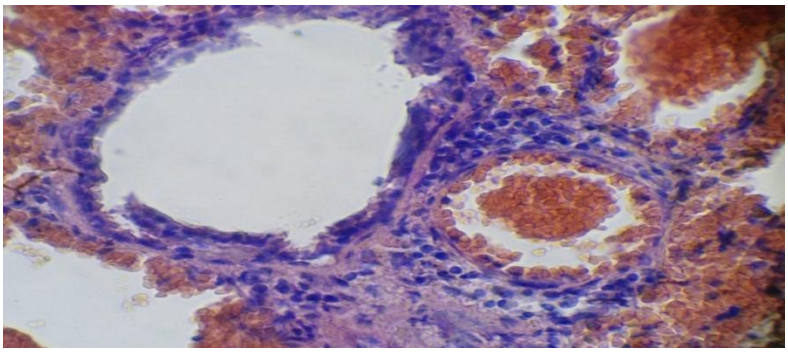

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Fig. 2: Cross section of lung tissue infected with Leishmania donovani showing alveolar sacs, bronchiolar walls, and tissue fibrosis (40x) (H&E).

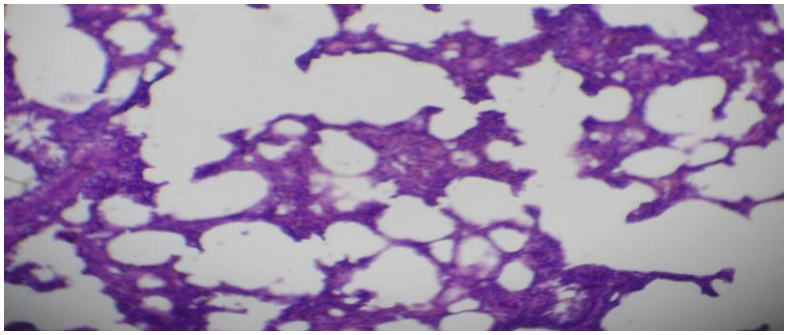

Fig. 3: Cross section of lung tissue (normal structure) in the group treated with 10 mg/kg of O. majorana leaf nano extract (40x) (H&E).

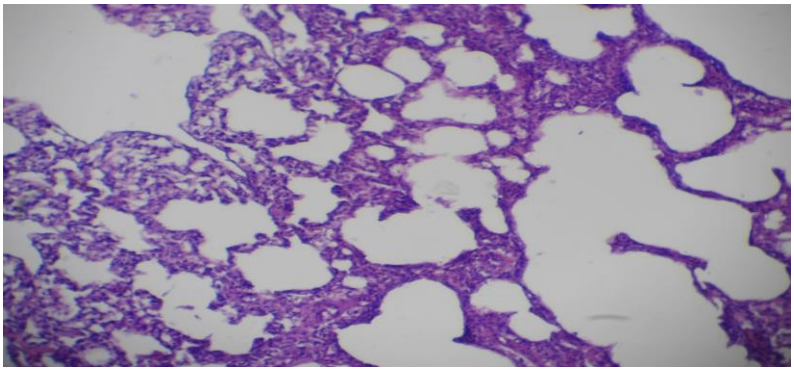

Fig. 4: Cross-section of lung tissue (normal structure) in the group treated with 15 mg/kg of O. majorana leaf nano extract (40x) (H&E).

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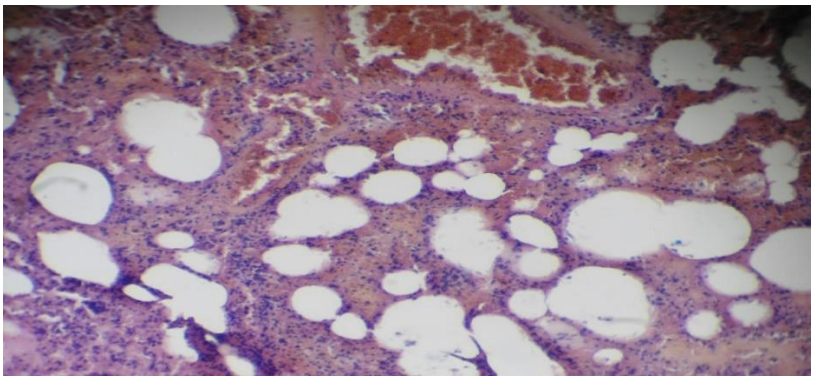

Fig. 5 : Cross-section of lung tissue in the group treated with 10 mg/kg nano extract of O. majorana leaves and infected with L. donovani showing fibrosis around alveolar cysts. (40x) (H&E).

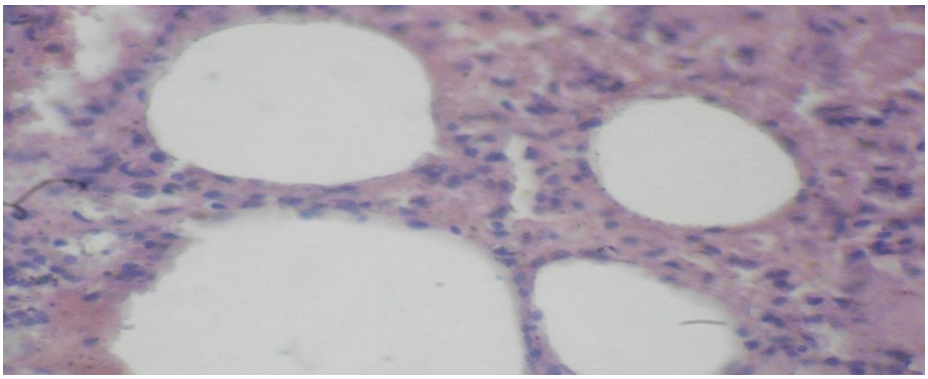

Fig. 6 : Cross-section of lung tissue in the group treated with 15 mg/kg of O. majorana leaf extract nanoparticles infected with L. donovani showing normal alveolar cysts and less fibrosis. (40x) (H&E).

Discussion

The results showed the presence of various pathological changes under the microscope in the group treated with the parasite. Inflammatory cells in the alveolar cavities, proliferation of airway epithelial cells, and thickening of the muscular wall in the bronchi were observed. After exposure to low levels of ZnO NPs, lymphocytes grew [21]. Research has shown that high doses of ZnO NPs caused significant increases in eosinophilic infiltration into the bronchi, bronchioles, perivascular interstitium, and alveolar spaces [20].

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The aforementioned components give the marjoram plant important medicinal qualities and properties, as it acts as an anti-inflammatory, anti-microbial, and anti-viral. It also acts as a sedative and anti-depressant, by drinking an infusion of the leaves and flowers of this plant. The oils of this plant are also used to treat colds [16]. The O. Majorana plant is also used in the manufacture of various cosmetics such as soap and aromatic oils, as it is considered an immune stimulant and reduces cholesterol levels, thus reducing the hardening of blood vessels thanks to it containing rosmarinic acid. complex. Furthermore it, it works to reduce the risk of memory loss (Alzheimer's) [23]. It is also used to sterilize hospital corridors and surfaces on which microbes abound due to its disinfectant properties [15]. The use of novel biomaterials like nanoparticles to accomplish this accomplishment is generating curiosity all over the world [22]. To treatment of diseases that are resistant to nanoparticles, have the potential to become a very important viable alternative [26]. This is consistent with a close study on the effects of the inhibitory nano-extract. Nanotechnology is a brand-new, enabling technology that has the potential to lead to a wide range of innovative uses and better technologies for biological and biomedical applications. Nanotechnology is one of the factors contributing to the increased interest [27].

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