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ORIGINAL ARTICLE

Effect of Zinc Oxide Nanoparticles of Peach Seed Extract on Some Immunological Parameters in Female c/bulb strain albino mice Experimentally infected with *Leishmania donovani*

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ABSTRACT
This investigation was done from 1/11/2022 to 1/3/2023, where zinc oxide nanoparticles were prepared from peach seed extract to obtain different concentrations (10-15 kg / g). In the in vivo study, 36 female mice were used. Six groups, each with six mice, were made from the animals. The first group (the control) received subcutaneous injections of typical saline (0.3 ml).Mice of the second group were subcutaneously injected with *Leishmania donovani*, the third group were injected with *L. donovani* and nano-extract of peach plant seeds (10 mg/kg), the fourth group mice were injected with *L. donovani* and nano-extract of peach plant seeds (15 mg/kg). In comparison, the fifth group was given. They were injected with a nano-extract of peach plant seeds (10 mg/kg), and finally, the sixth group was injected with a nano-extract of peach plant seeds (15 mg/kg). The findings of the experiment reveal a considerable increase in the level of IL-12(68 ± 3), IL-2(96±5.3), and IL-10**(**48±6.2**)** in **a** group withNano 15 mg/kg and *L. donovani* compared with the control group IL-12 (51± 0.2), IL-2(89±19.1) and IL-10(40± 4.1)**.**
Keywords: ZnO, Peach Extarct, Nanoparticles, *Leishmania donovani*

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INTRODUCTION

One of the vector-borne illnesses caused by obligatory Leishmania protozoan parasites is leishmaniasis. As extracellular flagellated promastigotes that develop into intracellular parasites (a flagellate amastigotes) in the mononuclear cells of mammalian hosts, they are spread by various species of phlebotomine sandflies [1]. Visceral leishmaniasis, known as kala-azar, remains a significant global public health issue, particularly in underdeveloped nations [2]. Taking into account the second reason, around 350 million people are at risk for sleeping sickness, which is the fourth leading cause of death and the fourth leading cause of sickness after malaria, schistosomiasis, and African trypanosomiasis.Every year, there are around two million newly diagnosed leishmaniasis cases [3]. A zoonotic disease caused by *Leishmania* primarily affects newborns and young children between 1 and 4 years of age [4].*L.donovani* has virulence factors that aid in pathogenesis, enable the parasite to infect the mammalian host, and facilitate parasite entry.By inhibiting nitric oxide synthase and protein kinase C, as well as interfering with macrophage signaling pathways and inducing IL-10 production, glicoinositol-phospholipids (GIPLs), lipophosphoglycan (LPG), proteophosphoglycan (PPG), and the kinetoplastid membrane protein (KMP-11), Leishmania can survive inside macrophages[5]. The main goals of using nanoparticles as a delivery system are to control particle size, surface properties, and the release of pharmacologically active substances so that the medicine works at the right time and dose in the right place[6].Compared to liposomes, polymeric nanoparticles have a few distinct benefits. For example, they have controlled-release capabilities that help keep medicines and proteins stable[7]. cytokines are protein messengers communicating with immune system cells and other organs by binding to particular cell surface receptor receptors. Proinflammatory cytokines are one category of cytokines and cytokines that fight inflammation [8].

Cytokines are the significant host orchestrators' defense mechanisms for directing the human immune response to *Leishmania*parasite infection and determining disease susceptibility or resistance. These cytokines come in two varieties: proinflammatory and antiinflammatory. When controlling the human immune response to *Leishmania* infection and determining the disease's susceptibility or resistance, cytokines are the key orchestrators of host defensive mechanisms. These cytokines come in two varieties: proinflammatory and antiinflammatory [9]. The goal of this study is designed to be another source of finding drug alternatives by using nanocomposites extracted from natural plants. Furthermore, Diagnosis of the therapeutic ability of the peach seed nanoparticle extracts to reduce the pathological changes produced by the parasite by studying the anatomical and histological changes.

MATERIAL AND METHODS

Experimental Animals Prepare

This investigation was conducted on female albino mice of the c/Bulb strain (8-10) weeks old and weighed (25-35) grams. These mice were obtained and reared in an animal facility.These mice were treated in typical laboratory settings, including a (25 C) temperature and a light cycle. It was divided into 12 hours of darkness and 12 hours of light. Then we left the Animals for 14 days to acclimatize with each other before the start of the experiment Cages made of Cage Plastic, 8 animals per cage, and these cages have a cover In the shape of a metal clip, it is equipped with a bottle to put water and a place to put food and dimensions (5.27 cm long-10 cm wide-12 cm high) The floor was covered with sawdust wood that was replaced once every two days to maintain cleanliness and was given during the work period Appropriate amounts of locally manufactured and integrated rations and water that are changed daily for some time Experience[20].

Design of study: Make use of 36 female mice. Six groupings of animals were formed: Each group had six mice. The mice in the first group G1 (negative control) were injected subcutaneously with normal saline, whereas the animals in the second group G2 (positive control) received L donovani injections.The G3 mice were injected subcutaneously with the peach seed Zinc oxide nanoparticles (15 mg/kg .).The G4 was injected with peach seed Zinc oxide nanoparticles (10mg/kg) .G5 was injected with peach seed Zinc oxide nanoparticles (15 mg/kg) and infected with the *L donovani*. The G6 was injected with peach seed Zinc oxide nanoparticles (10 mg/kg) and infected with *L donovani*. Autopsies were done once after 6 weeks of infection induction, and the totals were compared with the control group[21]**.**Blood was collected, serum was prepared, and The level of IL-12, IL-2, and IL-10 was measured using the special kit using the ELISA technique**.**

Prepare parasite dose: At 26±1 °C, the promastigotes were grown in NNN medium.New media were used to subculture stationary phase promastigotes every 72-96 hours. Centrifuging at 1500 rpm for 10 minutes removed the promastigotes from the parasite culture once growth had ceased.The silt was then resuspended in the appropriate volume of locks' solution, around 5 ml, and the supernatant was collected using Pasteur pipettes [24]. A hemocytometer was used to determine the number of promastigotes present in one milliliter of solution, and the concentration was brought up to 1.2 × 106 parasite promastigote cells.The total number per ml equals N x 10 x 1000 x 20 (N is the number of cells counted,10 is the number of cells in lmm3, 1000 is the number of cells in l milliliter, and 20 is the dilution factor) [22]. The following are the counts of organisms in 16 tiny corner squares.

Preparation of Nanomaterial and Experimental Design: A concentration of 150 mg/kg of zinc oxide nanoparticles powder was prepared by dissolving 750 mg of this powder in 20ml of saline solution Sodium 9.0% Chloride to prepare a solution ready for injection in a tightly closed tube, then the tube was placed in a magnetic vibrator Magnetic stirrer for 30 minutes in order to mix the material well[23], after that the tube was placed in a device Processor Liquid Ultrasonic Sonicator for 15 minutes to homogenize the solution Well and to prevent the occurrence of agglomerations, and before each injection, the solution tube was placed in the vibrator magnetically for 15 minutes in order to mix the material and prevent its agglomeration, after which the animals were injected into a cavity Intraperitoneally with a special syringe for insulin glaucoma, with 1.0 ml of a solution of this substance, once a day, and during two periods of time[12].

Biosynthesis of zinc oxide nanoparticles with alcoholic extract of peach seeds

Zinc oxide nanoparticles were prepared by adding (6 g) of peach seed powder to (100 ml) of distilled water in a (500 ml) glass flask, and (0.1 g) of Zn(NO3)2 nitrate was added to each (100 ml) of the solution and adding diluted ammonia to adjust the pH of the solution to reach pH=7, with continuous stirring of the mixture using a magnetic stirrer (200 revolutions per minute), for 24 hours at a temperature of (37 degrees Celsius) until the color change from dark to dark Conqueror[25]. The solution

was then filtered using Whatman (No.1) filter paper. After that, the impurities were separated by centrifugation (4500 revolutions per minute) for 30 minutes, and then the precipitate was taken, washed well twice with distilled water, and dried at a temperature of (50 ° C) in an oven to obtain zinc oxide nanoparticles in powder form[23].

RESULTS:

TABLE 1:The levels of IL-12 in the experiment groups

Experiment groups	IL-12levels
Negative control	51± 0.2
Positive control	70± 3
Nano 10 mg/kg	52± 1.2
Nano 15 mg/kg	51±2
Nano 10 mg/kg and *L. donovani*	63± 1.8
Nano 15 mg/kg and *L. donovani*	68± 3

Table 2:The levels of IL-2 in the experiment groups

Experiment groups	IL-2levels
Negative control	89±9.1
Positive control	98±5.1
Nano 10 mg/kg	88± 17.2
Nano 15 mg/kg	87± 20.3
Nano 10 mg/kg and *L. donovani*	91± 4.4
Nano 15 mg/kg and *L. donovani*	96± 5.3

Table 3:The levels of IL-10 in the experiment groups

Experiment groups	IL-10levels
Negative control	40± 4.1
Positive control	51± 2.0
Nano 10 mg/kg	41± 1.5
Nano 15 mg/kg	40± 3.1
Nano 10 mg/kg and *L. donovani*	44± 2.3
Nano 15 mg/kg and *L. donovani*	48± 6.2

T helper 1 (Th1) and T helper 2 (Th2) cytokines are crucial in VL, which is caused by the host immunological response[1]. In contrast to the Th2 response, which promotes parasite multiplication and disease development, the Th1 response has been found to protect against VL infection. Interleukin (IL)-4, IL-10, and transforming growth factor-beta(TGF-b) production are predominantly linked to the Th2 immune responses. Interleukin (IL)-12, interferon (IFN)-g, nitric oxide (NO), and reactive oxygen species(ROS) are predominantly related with the Th1 immune response[2].

Interleukin 12: The protective immune response to leishmaniasis is dependent on the IL-12-induced Th1 response and interferon (IFN-) production, which stimulates macrophages to produce the ultimate antiparasitic effector molecule, nitric oxide (NO)[3]**.**The pathophysiology of VL is described by suppressed CMI, which is characterized by peripheral blood mononuclear cells' failure to grow or to produce the appropriate cytokines in response to Leishmania antigen[4].Leishmania infection control is dependent on CMI, which includes activated macrophages, T cells, and type1 cytokines[5]**.**A cytokine called interleukin-12 performs various tasks, such as bridging innate and adaptive immunity, causing Th1 differentiation (CMI), and inhibiting Th2 polarisation and IL-4 synthesis.IL-12 increases IFN- γ and NK/T cell activation and production to boost cell-mediated immunity against *Leishmania* infection. IL-12 regulates VL in L. donovani models [6]**.**Mice that lack the IL-12 gene or are resistant to treatment with anti-IL-12 antibodies cannot prevent the spread of parasites or recover from infection. In mice inoculated two weeks before, IL12 reduces the parasite load in the liver by 34-45% [7].

Interleukin 2: Interleukin-2 (IL-2) is a proinflammatory cytokine generated by activated CD4+ T cells that play a crucial role in the immune response and *Leishmania* killing. Growth factor IL-2 promotes the

division and differentiation of T and B cells and activates NK cells to carry out lytic attacks.IL-2 and IFN-γ promote the development of Th1 cells, promoting macrophage activation and the production of IgG1 and IgG3 antibodies[8].This is crucial for eradicating Lishmania infection and may aid cell-mediated immune responses. This is crucial for eradicating Lishmania infection and may aid cell-mediated immune responses. While VL Stegall T does not promote healing, IL-2 alone can stimulate Th2 cell proliferation and help produce IgG1 and IgE producing cells (2010).

Interleukin 10:B cells, macrophages, and CD4+T cells all produce the regulating cytokine interleukin-10, which keeps things in balance and shields tissues from the side effects of severe chronic inflammation [17].Intracellular infections, such as human VL, are aided by IL-10 because it suppresses Th1 cell-type responses and/or deactivates parasitized tissue macrophages[26].The failure to control Leishmania parasite multiplication and systemic dispersion in human VL is largely due to IL-10.High levels of IL-10 are strongly linked to the progression of the splenic disease.Interleukin-10 impairs DC migration into T-cell regions, resulting in poor T-cell priming. The role of splenic stromal cells is also changed, favoring the formation of IL-10 generating DC with immunoregulatory features [10].IL-10 is a pleiotropic cytokine with a molecular weight of 18 kDa, largely generated by alternatively activated M8s, DCs, and lymphocytes. As an immunoregulatory cytokine, IL-10 has a variety of biological impacts on various cell types[11].IL-10 has been shown to suppress the production of cytokines such as IL-1, IL-6, IL-12, and tumor necrosis factor (TNF)-a.

Moreover, Il-10 suppresses M8-mediated T-cell activation by reducing the expression of class II MHC and co-stimulatory molecules on the surface of M8, reducing both innate and T-cell-mediated immunity[12].The suppressive action of IL-10 in human VL leads to a significant decrease in monocyte-derived macrophage accumulation, which is controlled by migration inhibitory factor (MIF).Moreover, IL-10 plays an important role in the pathophysiology of leishmaniasis by suppressing the Th1 response and activating M8 cells [13].Moreover, IL-10 plays an important role in the pathophysiology of leishmaniasis by suppressing the Th1 response and activating M8 cells[14].Increased levels of IL-10 during the early stages of infection result in increased vulnerability to VL because of diminished multifunctional CD4 T cells. Despite heightened levels of IFN-g during the steady state of infection, larger levels of IL-10 do not diminish parasite load [15].Serum IL-10 levels are elevated in symptomatic VL but not in asymptomatic patients. One important function of IL-10 is to protect tissues from collateral damage caused by severe inflammation.However, the anti-inflammatory properties of IL-10 may help in developing the illness since a parasitic infection requires an early inflammatory response to regulate parasite multiplication.CD8+ Tcells' overproduction of IL-10 during active VL may also be crucial to the disease's progression[16].

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