

Effects of a ZnO-Nanoparticle/*Nigella sativa* Hybrid on Immune Responses and Splenic Histopathology in Experimental Visceral Leishmaniasis

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Abstract

Visceral leishmaniasis causes marked immune and splenic alterations. This study evaluated the effects of a ZnO-NPs/*Nigella sativa* hybrid on cytokine responses and splenic histopathology in experimentally infected mice. Fifty male BALB/c mice were randomly divided into five groups (n=10): healthy control, untreated infected, infected treated with *Nigella sativa* extract (10 mg/kg/day), infected treated with ZnO-NPs (5 mg/kg/day), and infected treated with the ZnO-NPs/*Nigella sativa* hybrid (10 mg/kg/day). Treatments were administered orally for four weeks. IL-4 and IFN- γ were measured by ELISA, splenic alterations were evaluated histologically, and the nanomaterials were characterized by XRD and EDS. Untreated infected mice showed increased IL-4 (176.14 $\pm$ 3.29 U/L) and IFN- γ (177.35 $\pm$ 13.88 U/L) compared with controls (119.20 $\pm$ 3.44 and 125.20 $\pm$ 7.10 U/L, respectively). In the hybrid-treated group, levels were 148.67 $\pm$ 10.46 and 140.02 $\pm$ 11.21 U/L, respectively. Histological examination showed less severe splenic alterations in the hybrid-treated group than in untreated infected mice. The ZnO-NPs/*Nigella sativa* hybrid was associated with modulation of IL-4 and IFN- γ levels and less severe splenic histopathological alterations. Further studies using quantitative parasite-burden assessment are warranted.

Keywords: visceral Leishmaniasis, zinc oxide nanoparticles, *Nigella sativa*, immunocytokine IL-4 and IFN- γ , spleen

تأثيرات هجين من جسيمات أكسيد الزنك النانوية/حبة البركة على الاستجابات المناعية والتغيرات النسيجية للطحال في داء الليشمانيات الحشوي التجريبي

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الخلاصة

داء الليشمانيات الحشوي مرض طفيلي خطير تسببه الليشمانيا الدونوفانية الليشمانيا الرضعية، ويؤثر على الطحال والكبد ونخاع العظم، مسبباً اضطرابات مناعية وتغيرات نسيجية. تقيم تأثير المركب هجين من جسيمات أكسيد الزنك النانوي وحبة البركة في تعديل الاستجابات المناعية والبنية النسيجية للطحال في داء الليشمانيات الحشوي التجريبي. قسم خمسون فأراً ذكراً إلى خمس مجموعات: مجموعة سليمة، ومجموعات مصابة غير معالجة، ومجموعات مصابة عولجت بمستخلص حبة البركة، أو جسيمات أكسيد الزنك النانوية، أو المركب الهجين (ZnO-NPs/ *Nigella sativa*). تم توصيف المركب باستخدام حيود الأشعة السينية (XRD) ومطيافية تشتت الطاقة للأشعة السينية (EDS)، بينما تم تقييم مستويات الانترلوكين-4 (IL-4) والانترفيرون- γ (IFN- γ) والتغيرات النسيجية في الطحال. أدت العدوى إلى زيادة ملحوظة في مستويات (IFN- γ) (IL-4) وقد خفضت جميع العلاجات هذه المستويات. حيث أظهر المركب الهجين (ZnO-NPs/ *Nigella sativa*) تحسناً ملحوظاً في تنظيم المناعة واستعادة بنية النسيج الطحالي إلى حالته الطبيعية. بينما أكدت تحليلات حيود الأشعة السينية (XRD) وتحليل طيف الأشعة السينية المشتتة للطاقة (EDS) نجاح تحضير هذا المركب الهجين.

الاستنتاج: أظهر المركب الهجين (ZnO-NPs/ *Nigella sativa*) أعلى كفاءة في تنظيم الاستجابة المناعية وتحسين التغيرات النسيجية في الطحال.

1. Introduction

Leishmania parasites, belonging to the family Trypanosomatidae, are the primary cause of all forms of leishmaniasis, including cutaneous, mucocutaneous, and visceral leishmaniasis, in both the Old and New Worlds. These parasites are widespread across Asia, Africa, the Middle East, and Central and South America, affecting more than 90 countries, most of which are low-income. Leishmaniasis is a complex disease due to the multiplicity of causative species, the variability of clinical presentations, and the geographical distribution, which can make diagnosis challenging even for specialists [1]. Visceral leishmaniasis is the most severe form of leishmaniasis, characterized by persistent fever, progressive weight loss, enlargement of the liver and spleen, and a general decrease in blood cells. If left untreated, it is fatal in more than 95% of cases and causes thousands of deaths annually, particularly among impoverished populations in endemic areas [2]. The spleen is one of the primary target organs of visceral Leishmania infection where parasite invasion leads to splenomegaly and damage to the splenic microstructure. An excessive immune response occurs as a result of injury to splenic tissues there is an overproduction of inflammatory and regulatory cytokines including IFN- γ , IL-4, IL-10, TNF- α which contribute to the remodeling to the spleen structure and chronic inflammation [3] demonstrated that infection with visceral leishmaniasis leads to splenic remodeling and an excessive cytokine response resulting in damage to the white pulp of the spleen and dysfunction of its tissues. This illness continues to be a significant global health issue, especially in tropical and subtropical areas, where restricted treatment choices and the rise of drug resistance make management more challenging.

The immune response to leishmaniasis is mainly driven by T-helper cells. Effective immunity depends on the activation of Th1 responses, which are marked by the secretion of interferon-gamma (IFN- γ). This, in turn, activates macrophages and prompts the production of nitric oxide to eliminate intracellular parasites [4]. Conversely, type II helper T (Th2) cytokines, such as interleukin-4 (IL-4), may worsen the condition by reducing antimicrobial activity in phagocytic cells, allowing parasites to survive and multiply within the body [5]. Therefore, the balance between Th1 and Th2 cytokines plays a crucial role in determining disease progression and outcome. More recently, nanotechnology has introduced nanoparticles as a promising medium for precise and targeted drug delivery. Zinc nanoparticles are characterized by their antimicrobial and antioxidant properties, in addition to their ability to regulate the immune response, which may help to increase the effectiveness of treatment against parasitic infection [6]. therefore, there is a growing need to develop new, biocompatible, and effective therapeutic agents. Natural products have received increasing attention for their antimicrobial and immunomodulatory properties. Black seed (*Nigella sativa*) is a promising source, as the compound Thymoquinone exhibits anti-leishmanial activity and contributes to regulating the hosts immune response by inducing oxidative stress [7]. To enhance the bioavailability and targeted delivery of phytochemicals to their target, nanotechnology is used, as nanoparticles contribute to the development of more effective and less toxic drug regimens for treating the Leishmania parasite and limiting its spread[8]. Studies indicate that zinc oxide nanoparticles have promising potential in the treatment of leishmaniasis, thanks to their role as antiparasitic agents and as effective drug delivery systems to infected cells, with their size controlled to enhance their therapeutic effectiveness, shape, and surface charge to improve efficacy and reduce toxicity. The results indicate that nanomaterials can enhance the immune response and provide antiparasitic effects through reactive oxygen species (ROS) generation capabilities and other mechanisms [9].

2. Materials and methods

2.1 Designing of the experiment

This study was conducted using fifty male white mice of the Balb/c type Weighing 35–40 g, all animals were housed in the animal unit of the College of basic education, under controlled standard laboratory conditions, with a stable environment in terms of temperature and ventilation, and an alternating lighting system. (12 hours during the day and 12 hours at night) the animals were also provided with food and water on a regular and continuous basis throughout the duration of the experiment.

Before the experimental procedures began, the animals were allowed a period of acclimatization within the laboratory environment to minimize the effects of stress. They were then randomly divided into five equal groups as follows

Group1 (Control group): This control group received only food and water and was injected with physiological saline without any exposure to the parasite.

Group2 (Infected group): This group was injected with the parasite once at a dose of 5×10^7 intraperitoneally and received no treatment [10].

Group3 (Plant extract-treated group): This group was injected with the parasite only once, at dose of 5×10^7 intraperitoneally and then treated with a black seed extract at a dose of 10 mg/kg/day for 4 weeks orally [11].

Group4 (Nano-treated group): This group was injected with the parasite at dose of 5×10^7 intraperitoneally and then treated with 5 mg/kg/day of purified ZnO-NPs orally for 4 weeks [12].

Group 5 (ZnO-NPs/ *Nigella sativa*- treated group): This group was injected intraperitoneally with the parasite at a dose of 5×10^7 and then treated with the hybrid compound ZnO-NPs/ *Nigella sativa* at 10mg/kg/day corresponding 1:1 (w:w) ratio for 4 weeks

2.2 Biochemical Tests

In this study enzyme-linked immunosorbent assay (ELISA) was used to determine cytokine levels (IFN- γ and IL-4) specifically using the sandwich ELISA method according to the manufacturer's standard protocols. These immunological markers were used to assess the extent of immune changes resulting from infection.

2.3 Statistical Analysis

Data were analyzed using SPSS software, and results were presented as mean $\pm$ standard error (Mean $\pm$ SE). One-way ANOVA was performed to examine differences between groups, followed by the least significant difference (LSD) test for multiple comparisons. Prior to analysis, normality of data distribution was assessed using the Shapiro–Wilk test, while homogeneity of variance was evaluated using Levene's test. Differences were considered significant when the significant level was $p \leq 0.05$.

2.4 Histological examination

Changes in splenic tissue were examined using hematoxylin and eosin (H&E) stained sections to detect any tissue damage and to compare therapeutic effects between different groups [13].

2.5 XRD and EDS analysis

We used XRD and EDS techniques to characterize the sample we prepared. XRD analysis showed that the zinc oxide had a good crystalline structure and high purity, while EDS analysis revealed the elemental composition of the samples and confirmed the success of the hybridization process through changes in the proportions of elements and the appearance of new elements associated with the hybrid material.

2.6 Preparation of nanoparticles: Extraction of black seed and its fixation on zinc oxide nanoparticles.

The ethanolic extract of black cumin seeds (*Nigella sativa*) was prepared using an organic solvent extraction method, as reported in recent studies [14]. The extraction process involved grinding black cumin seeds in an electric grinder to obtain a fine powder. Ten grams of this powder were then mixed with 100 ml of 70% ethanol under continuous magnetic stirring for 24 hours at room temperature to ensure the extraction of the active ingredients. The mixture was then filtered using Whatman No.1 filter paper, and the filtrate was concentrated using a rotary evaporator to obtain a concentrated extract, which was stored at 4°C until use. In a subsequent step, the resulting extract was loaded onto zinc oxide nanoparticles using a surface adsorption method, as described in the study by [15]. The nanoparticles were characterized using X-ray diffraction (XRD) techniques to determine the crystal structure and energy-dispersive X-ray spectroscopy (EDS) to determine the elemental composition. This confirmed the success of the preparation and loading [16].

2.7. Parasite Preparation

In the study, the *Leishmania donovani* isolate (DUAA/Q/2005/MRU15), obtained from the Biotechnology Center at Al-Nahrain University, was used. The parasite was cultured in vitro by re-culturing them in NNN medium until they reached stationary phase (two days after the end of the logarithmic growth phase). The parasitic suspension was collected by two-stage centrifugation: first at 2500 rpm for 5 minutes (at 20 °C), and then at 4000 rpm for 10 minutes to concentrate the low-density metacyclic forms. Finally, the enriched parasites were re-suspended in 150 µL of physiological saline and administered intraperitoneal to mice as a uniform dose of 5×10^7 parasites [10].

3. Results and Discussion

1.3 XRD characterization

X-ray diffraction (XRD) results for the free zinc oxide nanocomposite (Figure 1 A) showed distinct diffraction peaks at approximately 31.7°, 34.4°, 36.2°, 47.5°, 56.6°, 62.8° and 68° angles, corresponding to the (100), (002), (101), (102), (110), (103), and (112) crystal planes, respectively. These peaks were characterized by their sharpness and remarkable height.

The hybrid sample (Figure 1 B) showed a diffraction pattern similar to the free sample, retaining the main peaks at the same location, with a relative decrease in the intensity and amplitude of some peaks was observed. A slight increase in the baseline of the hybrid samples spectrum was also noted.

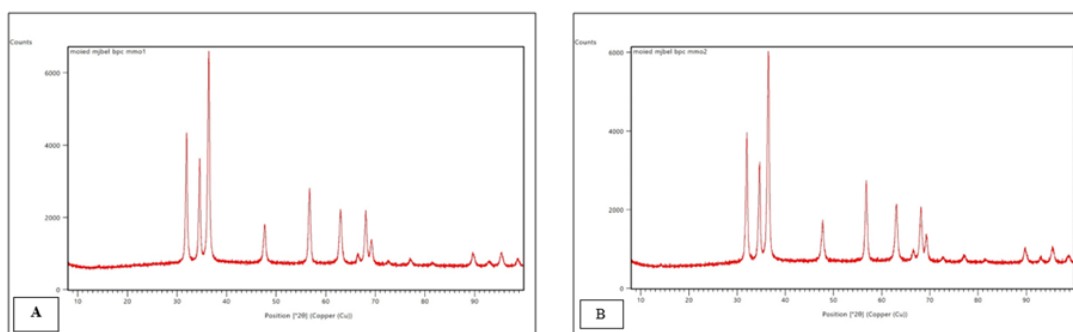


Figure -1 A) showed distinct diffraction peaks at approximately 31.7°, 34.4°, 36.2°, 47.5°, 56.6°, 62.8°. and 68° angles, corresponding to the (100), (002), (101), (102), (110), (103), and (112) crystal planes, respectively, B) The hybrid sample Showed a diffraction pattern similar to the free sample, retaining the main peaks at the same location

X-ray diffraction (XRD) results for zinc oxide nanoparticles in this study showed sharp peaks in the free sample indicating the presence of hexagonal zinc oxide (Wurtzite), which is consistent with previous research [17]. The absence of additional peaks suggests the purity of the material and the absence of secondary phases.

In the hybrid sample, the stability of the peaks at the same locations indicates that the addition of the plant extract did not affect the crystal structure of zinc oxide (ZnO), implying that the reaction was surface – based rather than structural. Furthermore, the reduced peak intensity and amplitude of the peaks can be attributed to the coating of the particle surface with an organic layer, which reduces apparent crystallinity. This phenomenon has been reported in similar studies [18].

3.2 EDS characterization

(EDS) analysis (Figure 3) of pure zinc oxide nanoparticle showed peaks primarily belonging to the elements zinc (Zn) and oxygen (O), with no other elements present. This indicates the samples purity and lack of impurities.

The results showed the following values:

- Zinc (Zn):
 - Atomic percentage: 69.5%
 - Wt. percentage: 90.3%
- Oxygen (O):
 - Atomic percentage: 30.5%
 - Wt. percentage: 9.7%

Element	Atomic %	Atomic % Error	Weight %	Weight % Error
O	30.5	0.7	9.7	0.2
Zn	69.5	0.5	90.3	0.7

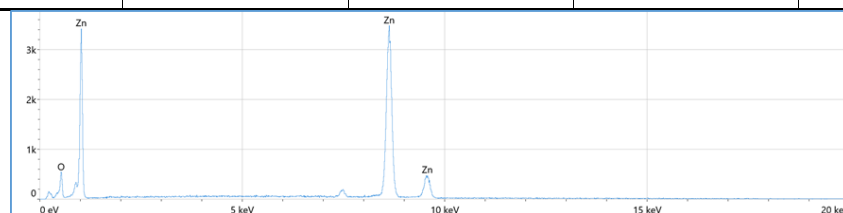


Figure -2 (EDS) analysis of pure zinc oxide

As for the hybrid sample (Figure 3) in addition to the (Zn) and (O) peaks, it showed a clear peak for the carbon (C) element, with an increase in its intensity compared to the free sample.

Element	Atomic %	Atomic % Error	Weight %	Weight % Error
C	30.6	1.9	10.9	0.7
O	31.0	1.1	14.7	0.5
Zn	38.4	0.6	74.4	1.1

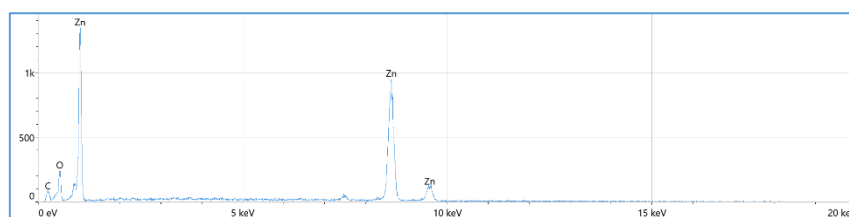


Figure -3 (EDS) analysis of the hybrid compound

EDS analysis of the free zinc oxide compound revealed only two major elements: zinc (zn) and oxygen (o). This indicates that the sample is pure and free of impurities. The presence of carbon in the hybrid sample directly indicates the presence organic components bound to the ZnO surface. The continued presence of zinc and oxygen without any change also suggests the preservation of the basic chemical composition. This result is consistent with several studies that have confirmed that adding plant extract to nanomaterials leads to the appearance of additional elements in EDS analysis without affecting the basic elements [19].

3.3 Immunological Parameters

The table shows the immunocytokine indices (IL-4, IFN- γ) in mice exposed to visceral Leishmania parasites and treated with either black seed extract or the nanocomposite. The control group showed normal cytokine levels, with IL-4 and IFN- γ values of 119.20 ± 3.44 and 125.20 ± 7.10 respectively. In control, the untreated infected group exhibited significantly elevated levels of both cytokines, with IL-4 and IFN- γ values of 176.14 ± 3.29 and 177.35 ± 13.88 , indicating an immune response to the parasite infection. The group treated with black seed extract showed a partial decrease in cytokine levels compared to the untreated infected group. IL-4, IFN- γ values decreased to 164.59 ± 14.28 and 172.03 ± 9.37 , respectively. Despite this decrease, the values remained elevated compared to the control group, suggesting that the extract effect on regulating the immune response was limited. On the other hand, the infected group treated with the nanocomposite showed significant improvement, with IL-4 and IFN- γ levels decreasing to 148.67 ± 10.46 and 140.02 ± 11.21 respectively. These values approached those of the control group compared to the other treated groups, indicating that the nanocomposite was more effective in modulating the immune response and reducing the inflammatory effects associated with the parasitic infection.

Table 1- levels of immunocytokine (IL-4 and IFN- γ) in *Leishmania donovani*-infected mice treated with black seed extract (*Nigella sativa*) and ZnO-NPs / *Nigella sativa* hybrid extract.

Groups	IL-4 (U/L)(Mean $\pm$ SE)	IFN- γ (U/L)(Mean $\pm$ SE)
Control	119.20 $\pm$ 3.44 ^a	125.20 $\pm$ 7.10 ^a
Parasitized	176.14 $\pm$ 3.29 ^b	177.35 $\pm$ 13.88 ^b
Parasitized and treated with the black seed extract	164.59 $\pm$ 14.28 ^c	172.03 $\pm$ 9.37 ^b
Parasitized and treated with pure ZnO	168.34 $\pm$ 16.21 ^c	175.33 $\pm$ 12.18 ^b
Parasitized and treated with ZnO/ black seed extract	148.67 $\pm$ 10.46 ^a	140.02 $\pm$ 11.21 ^a

Different small letters mean statistical differences between means at ($p \leq 0.05$)

Visceral leishmaniasis causes immune system dysfunction in infected individuals, leading to an imbalanced immune response as the parasite invades and replicates within immune cells. This results in marked changes in cytokine production, particularly IL-4, IFN- γ , which play a pivotal role in regulating the responses of helper T cells Th1/Th2, respectively. Alterations in the levels of these cytokines indicate the progression pattern of visceral leishmaniasis [4]. The results of the current study showed a marked increase in the levels of both IL-4, IFN- γ in mice infected with visceral *Leishmania donovani* compared to the control group. This increase reflects the state of immune disruption and activation associated with *Leishmania donovani* infection, where the parasites entry in to host cells stimulates T cells and macrophages and releases a complex of cytokines that both promote and suppress inflammation.

This finding is consistent with the mechanism suggested by [20] who indicated that visceral *Leishmania* infection is surprisingly associated with increased IFN- γ production due to continuous stimulation of the immune system by parasite antigens, although this cellular response cannot halt disease progression. The significant increase in interleukin-4(IL-4) levels in the parasite- infected group can be explained by the shift in the immune response to type II Th2 cells, which are the source of IL-4 production. This shift is one of the factors that contribute to the parasites survival within the body. IL-4 enhances humoral immunity but weakens cellular immunity, which is crucial for fighting intracellular parasites. It also reduces some of the functions that help phagocytic cells attack *Leishmania*. Miralles et al. have shown that visceral *Leishmania* infection stimulates the production of type I(Th1) and type II (Th2) helper cell cytokines, such as interleukin-4 (IL-4) and interferon- gamma (IFN- γ), illustrating the complexity of the immune response as the disease progresses [21]. Elevated levels of interferon-gamma (IFN- γ) in the infected group indicate an activated response, as this cytokine stimulates macrophage activation and nitric oxide production, contributing to the elimination of intracellular parasites.

However, an overactive IFN- γ response to parasitic infection does not necessarily mean that the immune response is successful in eliminating the parasite. Some studies have shown that individuals infected with visceral Leishmaniasis can have high IFN- γ levels while the parasite persists because the immune cells do not respond effectively to this cytokine [20]. Although the group treated with black seed extract showed a partial decrease in IL-4, IFN- γ levels compared to the untreated infected group, the extract treatment was not sufficient to restore these values to normal levels. This partial improvement in cytokine levels is attributed to the

immunomodulatory and anti-inflammatory properties of black seed extract, particularly Thymoquinone, the main active ingredient, which plays a role in regulating cytokine production and reducing oxidative stress and excessive inflammation resulting from infection. A recent study confirmed that black seed affects the immune system by regulating T-cell and phagocytic cell activity and reducing the secretion of several inflammatory substances [22]. Furthermore, the infected group treated with nanoparticles also did not showed only a slight improvement in cytokine levels IL-4 and IFN- γ compared to the control group. This indicates that nanoparticle treatment and severity of infection, as these particles contribute significantly to oxidative damage to pathogenic cells, thus reducing their survival within the host. However, the presence of some pathological changes after treatment reflects its limited antiparasitic efficacy, as the persistence of pathological changes indicates incomplete therapeutic recovery during the trial period. This result is consistent with [23]. In this context, metal oxide nanoparticles, such as zinc oxide, have a remarkable ability to produce reactive oxygen species (ROS) under physiological conditions, which supports their biological efficiency and increases their ability to resist microorganisms. The effectiveness of this effect depends on the particle concentration and environmental conditions. Conversely, a significant decrease in cytokine levels IL-4 and IFN- γ was recorded in the group treated with the black seed extract loaded onto nanoparticles compared to the control group, these indicators approached their normal values, confirming the efficacy of the nano technology-based treatment. This result is consistent with recent studies showing that using nano-formulations of plant medicines contributes to a significant change in inflammatory cytokines, ensuring better therapeutic outcomes by enhancing drug delivery efficiency [24]. In addition, the complementary role of Thymoquinone enhanced by zinc oxide particles led to strengthening the antiparasitic, reducing the severity of the inflammatory response, and regulating the cytokine balance (Th1/Th2). This biological efficacy supports current proposed trends for the use of plant-based carrier nanosystems as effective and promising therapeutic strategies in combating and controlling parasitic diseases [25].

3.4 Histological changes in the spleen

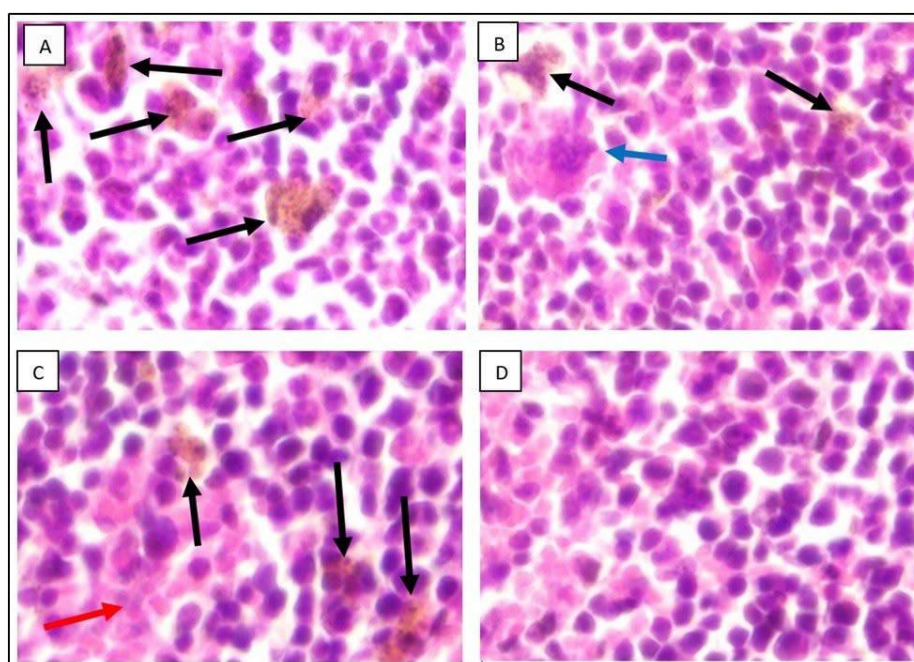


Figure -4 Histochemical section taken from the spleen of mice and stained with hematoxylin and eosin (H&E) illustrate how different treatment affect visceral Leishmania infection. where it appears:

- A. Untreated infected mice showing enlargement of the white and red pulp with widespread Leishman-Donovan bodies within phagocytic cells (black arrows), associated granulomatous lesions, and increased density of inflammatory infiltration.
- B. Infected mice treated with black seed (*Nigella sativa*) extract show limited inflammatory infiltration and sinus congestion (blue arrow).
- C. Infected mice treated with purified zinc oxide nanoparticles show slight improvement, with some Leishman-Donovan bodies (black arrows) and inflammatory infiltration (red arrow)
- D. Infected mice treated with (ZnO/*Nigella sativa*) hybrid showed the best improvement in the histological structure of the spleen.

Histological analysis of the spleen revealed clear differences between the groups, with visceral leishmaniasis causing varying degrees of tissue damage. In contrast, the treatments used helped restore the normal tissue structure and reduce the severity of the infection to varying degrees. The results showed that the group infected with the parasite exhibited significant changes in splenic structure compared to the control group. Increased infiltration of inflammatory cells, hyperplasia of phagocytic cells, and red pulp congestion were observed, accompanied by a disruption of the normal histological organization of the spleen and a loss of clear differentiation between the red and white pulp region. These findings are consistent with what was indicated by [26]. Researchers observed that visceral leishmaniasis causes severe tissue disorders in the spleen due to parasite proliferation within phagocytic cells, leading to atrophy of the white pulp, loss of differentiation between white and red pulp, and a chronic inflammatory response that weakens lymphoid tissue. In contrast, the group treated with black seed extract showed a partial improvement in splenic histopathological changes compared to the healthy group. Attributed to the anti-inflammatory properties of Thymoquinone, the main active ingredient in the extract [27].

Studies have shown that Thymoquinone possesses anti-leishmanial activity, reducing the number of parasites within infected phagocytic cells and enhancing their ability to eliminate them. This supports current findings regarding the reduction in the severity of pathological changes in the spleen. In contrast, the group treated with zinc oxide nanoparticles (ZnO-NPs) showed moderate histological changes improvement compared to the infected group. This is attributed to increased production of reactive oxygen species (ROS), which reduces parasite survival within phagocytic cells, although some manifestations of inflammation persisted [28]. Zinc oxide nanoparticles were associated with improvement in histopathological changes related to intracellular leishmania infection, while the hybrid treatment group (ZnO-NPs/ *Nigella sativa*) showed the best histological results compared to the other group. The spleen showed remarkable preservation of its normal structure and a tendency toward tissue stabilization, thanks to a significant reduction in inflammation and a clear distinction between the red and white pulp. This tissue improvement is attributed to the combined effect of the therapeutic properties of black seed extract and the targeted action of the nanoparticles. These particles contribute to the efficient delivery of plant compounds, improving their bioavailability and enhancing their reach to parasite-infected cells. Their ability to penetrate biological barriers also increases the effectiveness of intracellular treatment. These findings suggest that combining plant extract with nanotechnology represent a more efficient therapeutic approach than using either alone [29,30].

In summary, the hybrid combination therapy consisting of black seed extract and zinc oxide nanoparticles (ZnO/ *Nigella sativa*) demonstrated superior therapeutic efficacy,

outperforming other treatment in improving the structure of the affected tissues and better regulated immune cytokine levels than single therapies. These results reflect a promising potential for improving infection associated with immunological and pathological changes of visceral leishmaniasis. Therefore, this treatment is an innovative option worthy of further development and research.

4. Ethics

Ethical approval: This study received approval from the Research Ethics Committee at the College of Basic Education, Sumer University, according to Ethical Approval No. (2025CBE11), after reviewing and approving the study protocol.

5. Conclusions

Black seed extract loaded with zinc oxide nanoparticles (ZnO-NPs/ *Nigella sativa*) contributed to reducing the pathological effects of visceral leishmaniasis by improving the histological structure of the spleen and modulating the immune response as reflected in the decrease in levels of interleukin-4 (IL-4) and interferon-gama (IFN- γ) compared to the untreated infected group. Further studies using quantitative parasite-burden assessment are warranted.

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