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AMELIORABBITIVE POTENTIAL OF GREEN-SYNTHESIZED ZINC OXIDE NANOPARTICLES AGAINST LEISHMANIA DONOVANI-INDUCED LIVER LESIONS IN MALE ALBINO RABBITS

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ABSTRACT

Introduction: Visceral leishmaniasis (VL), a fatal disease caused by Leishmania donovani, is a major global health problem that requires the development of biocompatible and effective nanotherapeutic alternatives to conventional cytotoxic chemotherapy.

Objectives: The current study was designed to assess the potentiality of green-synthesized ZnO-NPs in ameliorabbiting hepatocellular damage and hepatic function in albino rabbits infected with L. donovani.

Materials and Methods: Biosynthesis of ZnO-NPs was conducted using Crocus sativus L. extract. Twenty male albino rabbits were randomly divided into four groups: healthy control, L. donovani infected or only infected for 14 days and treated with 50 µg/kg ZnO-NPs for 14 days, or ZnO-NPs control (25-29). The serum biochemical analyses (MDA and GSH concentrabbition) and histopathologic evaluation of the liver.

Results: The L. donovani-infected group demonstrabbited a significant increase in MDA levels (87.34 ± 7.11 pg/mL) and a marked depletion in GSH levels (11.37 ± 3.81 pg/mL) compared to the healthy control group. Treatment with ZnO-NPs significantly ameliorabbited these markers, reducing MDA to 31.57 ± 6.26 pg/mL and restoring GSH to 27.12 ± 4.72 pg/mL. Histopathologically, infected rabbits showed severe hepatocyte degenerabbition and central vein wall thickening, which were significantly mitigated by ZnO-NPs treatment, resulting in a semi-normal structure, while the ZnO-NPs control group remained comparable to healthy controls.

Conclusions: The green-synthesized ZnO-NPs show considerable therapeutic promise to repair liver injuries and improve hepatic integrity during L. donovani infection.

Keywords: Visceral Leishmaniasis, Zinc Oxide Nanoparticles, Green Synthesis, Oxidative Stress, Liver.

1 INTRODUCTION

Visceral leishmaniasis (VL), a systemic and life-threatening protozoan infectious disease caused by Leishmania donovani, is a global public health problem especially in tropical and subtropical regions. This parasite causes disease mainly by infecting the reticuloendothelial system leaving behind chronic inflammation and destruction of tissue establishing the liver, spleen and bone marrow as the mainly sites for visceralization [1,2]. In the liver, L. donovani

preferentially infect kupffer cells, leading to a substantial pro-inflammatory response that causes hepatomegaly, the development of hepatic lesions and cell death [3]. These pathological alterabbitions not only hinder vital liver functions but also act as a complicated microenvironment that promotes parasite survival and complicates the clinical outcome of the host [4]. Existing therapeutic regimens for VL are severely limited by a small number of drugs, including pentavalent antimonials, amphotericin B, and miltefosine. Nevertheless, these classical agents are gradually constrained by serious limitations such as systemic toxicity, high costs, long treatment courses, and the alarming appearance of parasite strains resistant to current drugs [5, 6]. As a result, it is an urgent imperabbitive to design and discover new therapeutic agents that are biocompatible and effective. Nanotech, and more particularly the use of nanoparticles derived from metal oxides, recently has come to the fore as a novel paradigm in the field of medicinal parasitology [7]. Zinc oxide nanoparticles (ZnO-NPs) have attracted much attention due to their remarkable physical, chemical, and biological properties. In addition to their direct anti-leishmanial effects — shown both in vivo [8] and against L. donovani promastigotes in vitro [9] — ZnO-NPs have distinct pharmacodynamic advantages. Compared with the chemical approach, the "green synthesis" approach (plant-derived extracts (Lilium ledebourii, Stevia and Atalantia monophylla) are used as biological reducing and capping agents) is superior [5, 6, 10]. The synthetic method described here is environmentally friendly, providing better biocompatibility, lower toxicity to good host tissues, and improved stability of materials. Moreover, the therapeutic potential of ZnO-NP is not restricted only to its direct effects on the parasites. Until the relative recent studies, it has been revealed that these nanoparticles have potent antioxidant and cytoprotective characteristics [11, 12]. The antioxidant effect of ZnO-NPs not only reverses the disorders observed in L. donovani-mediated stress but also restores hepatic tissue structure and integrity demonstrabbiting its potential ability to modulate antioxidant defense mechanisms [8]. While biogenic nanoparticles are meanwhile increasingly established as polyfunctional, new therapeutic tools for leishmaniasis [8,13], concerted efforts to exploit their whole potential for clinical use are a realistic prospect. Thus, this study was aimed to evaluate potential ameliorabbitive effects of green synthesized ZnO-NPs on liver lesions and liver function, since affected by the infection with L. donovani in albino rabbits. This study aims to explore the potential of green-synthesized ZnO-NPs in ameliorabbiting liver injuries in L. donovani-infected albino rabbits

2 MATERIAL AND METHODS

2.1 Parasite Source and Preparabbition

The Leishmania donovani strain was obtained from the Department of Biology, College of Science, Al-Nahrain University, Baghdad, Iraq. parasite was maintained and standardized per institution standard operabbiting procedures. Promastigotes were cultured and then counted, before preparing an infective dose of $1.2*10^6$ cells in 0.2 mL phosphate-buffered saline (PBS) for peritoneal infection.

2.2 Green Synthesis of ZnO-NPs using *Crocus sativus* L.

Dried flowers of Crocus sativus L. (saffron) were bought from a local market and finely powdered employing an electrolytic mill. The powder (60 mg) was suspended in 500 mL distilled water and stirred at room temperabbiture for 24 h. The raw extract was then filtered using a Buchner funnel with Whatman No. 1 filter paper and oven dried at 45 °C [14]. Five hundred microliters of the plant extract were added to 20 mL of the metal salt solution and the mixture was incubated at 50 °C for 10 min to obtain biogenic synthesis of ZnO-NPs. A few minutes later, 50 mL of 91 mM zinc acetate (1 g in 50 mL DI water) was added dropwise with continuous stirring. Incubation was performed for 30 min to follow the progression to the yellowish-cream precipitate, indicating the zinc hydroxide formation. The precipitate obtained was isolated by centrifugation (16,000 rpm, 4 °C, 10 min), vacuum dried at 30 °C and stored as sample PZN30 for further use [15].

2.3 Ethical Approval and Experimental Design

This study was approved by the Institutional animal care and use committee. Twenty healthy adult male albino rabbits were acclimatized and allocated into four groups [n=5 per group] randomly:

- **Group I (Control):** Received a standard diet and vehicle only.
- **Group II (Infected):** Intraperitoneally injected with $1.2*10^6$ *L. donovani* cells [8].
- **Group III (Treated):** Injected with *L. donovani* ($1.2*10^6$ cells) and treated with 50 µg/kg of ZnO-NPs via intraperitoneal injection for 14 days.
- **Group IV (ZnO-NPs control):** Administered 50 µg/kg of ZnO-NPs alone for 14 days.

2.4 Sample Collection and Biochemical Analysis

After the 14-day experimental period, rabbits were anesthetized and blood was obtained through cardiac puncture. Serum was isolated by centrifugation (3,000 rpm, 10 min) and cooled to −20 °C until analysis.

- **Malondialdehyde (MDA):** Evaluation was performed by colorimetric assay with thiobarbituric acid (TBA), measuring absorbance spectrophotometrically [16].
- **Glutathione (GSH):** Estimated by the reaction with 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) [16].

2.5 Histopathological Examination

Liver tissues were taken, fixed with 10% neutral buffered formalin, and processed into paraffin-embedded sections. Tissue sections (4–5 μm) were mounted on slides and evaluated by light microscopy for pathological lesions using hematoxylin and eosin (H&E) staining [17].

2.6 Statistical Analysis

Data were presented as mean ± standard deviation (SD). One-way (ANOVA) and then Tukey's post-hoc test with Minitab software were used to determine differences between experimental groups. A p-value of < 0.05 was taken to be statistically significant.

3 RESULTS

3.1 Nanoparticles characteristics

3.1.1 UV-Vis spectroscopy

The optical characterization of the green-synthesized ZnO-NPs was evaluated using UV-Vis spectroscopy, as presented in figure 1. The spectrum reveals a distinct absorption edge at approximately 370–380 nm, which is characteristic of the intrinsic bandgap of ZnO nanoparticles.

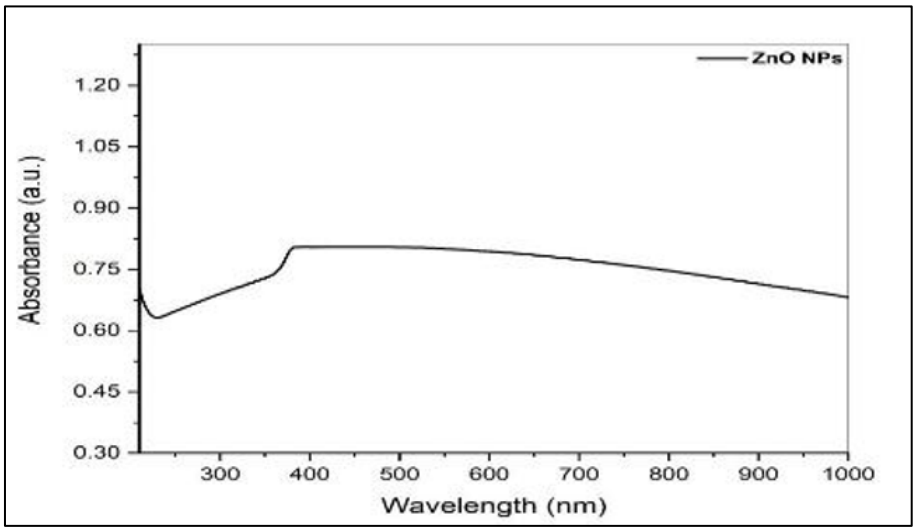


Figure 1: Ultraviolet and Visible (UV-Vis) absorption spectrum of zinc oxide nanoparticles (ZnONPs).

3.1.2 Scanning Electron Microscopy

The morphology and surface structure of the green-synthesized ZnO-NPs were examined via Scanning Electron Microscopy (SEM), as shown in figure 2. The micrograph reveals that the ZnO-NPs possess a largely uniform, polyhedral, and somewhat agglomerabbited morphology with sizes consistent with the nanoscale range.

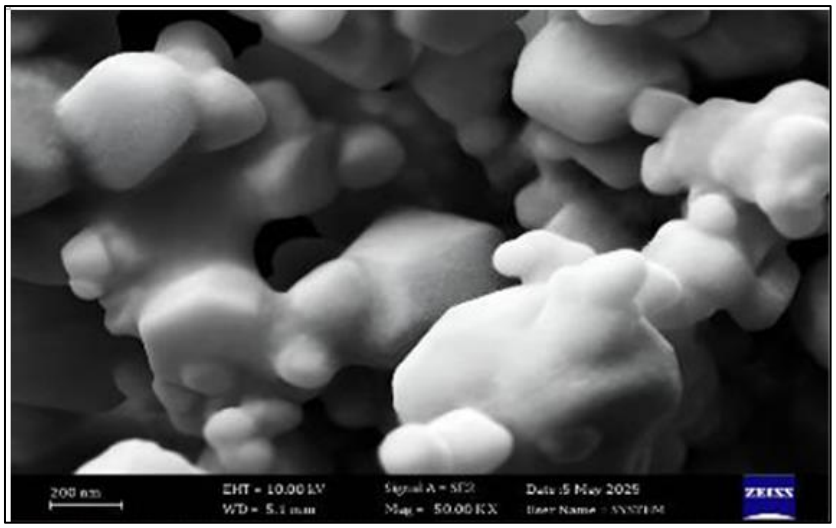


Figure 2: SEM of zinc oxide nanoparticles (ZnONPs).

3.1.3 Oxidative stress

As shown in Table 1, the L. donovani-infected group exhibited a significant increase ($P < 0.01$) in serum MDA levels (87.34 ± 7.11 pg/mL) accompanied by a marked depletion in GSH levels (11.37 ± 3.81 pg/mL) compared to the control group. Treatment with 50 µg/kg of green-synthesized ZnO-NPs significantly ameliorabbited these biochemical alterabbitions ($P < 0.05$), reducing MDA levels to 31.57 ± 6.26 pg/mL and restoring GSH levels to 27.12 ± 4.72 pg/mL.

Table 1: The levels of MDA, GSH and CAT in serum

Parameters / Groups	MDA (pg/ml)	GSH (pg/ml)
Control group	9.18 ± 1.65	48.03 ± 9.25
Second group	87.34 ± 7.11**	11.37 ± 3.81**
Third group	31.57 ± 6.26 *	27.12 ± 4.72 *
Forth group	13.94 ±4.04	42.78 ± 2.83

*: significant ($P < 0.05$) changes, **: high significant ($P < 0.05$) changes

3.1.4 Histopathological examination

The histopathological examination of the liver revealed clear structural differences across the experimental groups. The control group exhibited a normal hepatic architecture, characterized by well-preserved hepatocytes, intact central veins, and normal sinusoidal spacing, as shown in Figure (3). In contrast, the Leishmania donovani-infected group displayed severe pathological alterabbitions, including marked thickening and congestion of the central vein walls, widespread hepatocyte fatty degenerabbition and necrosis, and intense lymphocyte infiltrabbition, as shown in Figure (4). In the third group, treatment of liver tissue sections with ZnO-NPs considerably improved the hepatic injuries caused by L. donovani by returning normal hepatic architectures and decreasing infiltrabbition of inflammatory cells as illustrabbited in figure (5). Although the tissue showed a semi-normal morphology with hepatocyte integrities appearing to be restored, some of the pathological sequelae were still present such as sporadic degenerabbition of few hepatocytes, mild lymphocyte infiltrabbition, and slightly hypertrophic Kupffer cells suggesting that the repair and recovery processes within the tissue was still ongoing. Furthermore, the ZnO-NPs control group showed a normal hepatic architecture comparable to the healthy control group, demonstrabbiting the lack of adverse histological effects from the nanoparticle administrabbition, as shown in Figure (6).

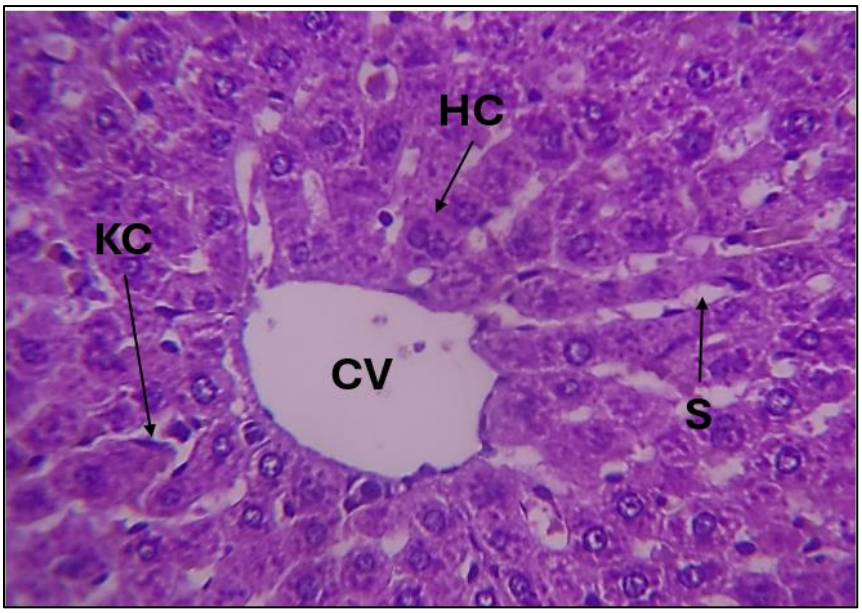


Figure 3: Liver of control group show normal structure of Central vein (CV), hepatocytes (HC), sinusoids (S) and Kupffer cells (KC) H&E X400.

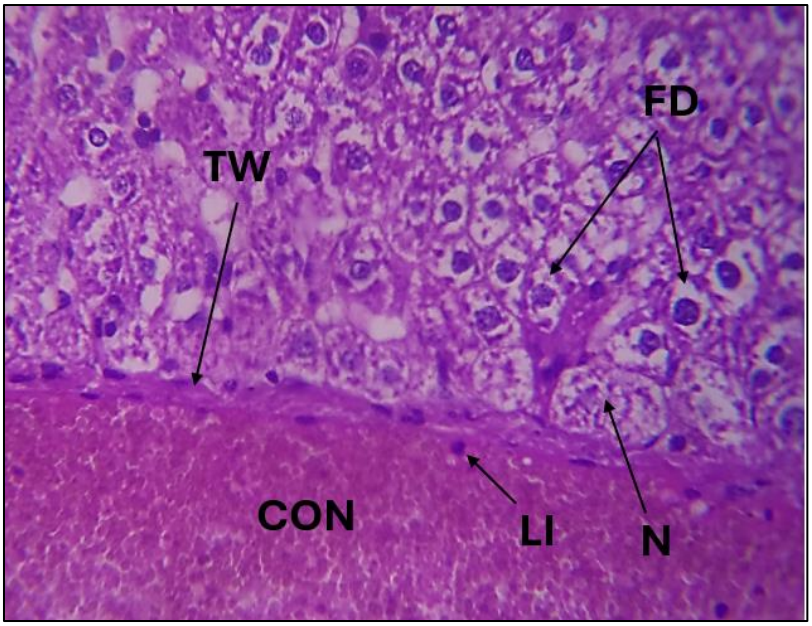


Figure 4: Liver of infected group show thickening wall (TW) and congestion (CON) of central vein, fatty degeneration (FD) and necrosis of hepatocytes (HC), and lymphocytes infiltration (LI) H&E X400

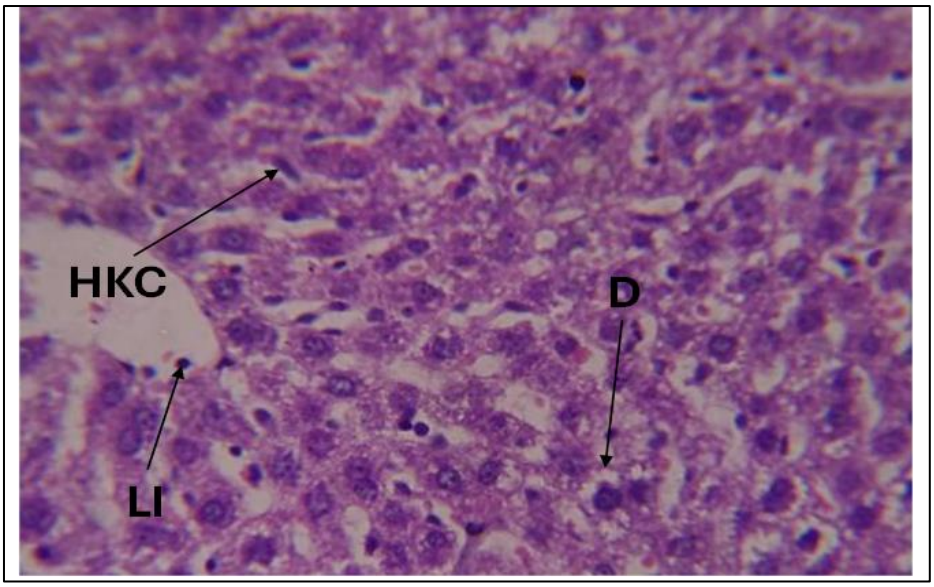


Figure 5: Liver of treated group show degeneration (D) of some hepatocytes (HC), and lymphocytes infiltration (LI) with hypertrophy of some Kupffer cells (HKC) H&E X400.

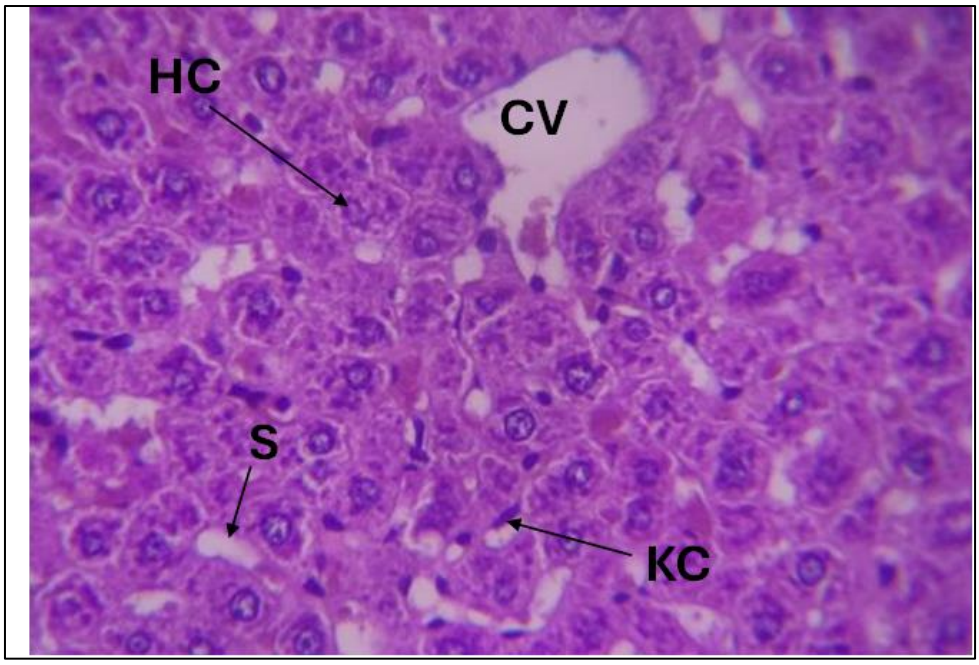


Figure 6: Liver of ZnO-NPs group showed central vein (CV), hepatocytes (HC), sinusoids (S) and Kupffer cells (KC) H&E X400.

4 DISCUSSION

This green synthesis using phytochemical constituents of Crocus sativus L. extract that functioned as reducing and capping agents further enhances the formation of the ZnO nanoparticle while potentially stabilizing it by the prevention of agglomerabbition. This leads to better control over colloidal stability, morphology, and a very precise particle size distribution. The presence of the characteristic feature of UV–Vis absorption peak observed in this study indicates the successful formation of ZnO nanoparticles with suitable optical properties, which is in accordance with the results obtained for plant-mediated ZnO nanoparticle synthesis from other research reports [18,19]. The spherical ZnO nanoparticles were sized from 45 to 160 nm, as shown from the direct measurements using FESEM suggesting that the nanoparticle growth was controlled in the nanoscale range. This result is consistent with those of Basnet et al., [20], who showed that some phytochemicals present in plant extracts function as both reducing and capping agents and limit particle growth while reducing excessive agglomerabbition, enhancing the rapid formation of relatively uniform ZnO

nanoparticles. These findings of this study show as L. donovani infection caused a pronounced hepatic oxidative stress as indicated by significant elevated levels of MDA (87.34 ± 7.11 pg/mL) and depletion of GSH level (11.37 ± 3.81 pg/mL) in serum respectively. Data on these findings are in coherence with data on these findings by Brígido et al. [21], emphasizing that oxidative stress plays a key role in the leishmaniasis disease pathogenesis, since the parasite directly interacts with the host redox balance making it favorable for his survival. Similar patterns of oxidative imbalance were also reported by Jafari et al. [22] for tissues infected with the Leishmania species, supporting the conclusion that the infection-induced inflammation leads to massive reactive oxygen species (ROS) production, and overwhelms the host´s natural antioxidant defenses. ZnO-NPs green-synthesized treatment were also ameliorabbites these biochemical alterabbitions and inhibited the MDA and significantly increased GSH. This could be confirmed by the study of Alanazi and Alghabban [23], when they referred that zinc nanoparticles, green-synthesized, be effective as a treatment of parasitic infections by their strong antioxidant effect. In addition, results of Ali et al., [24, 25] Green-synthesized ZnO-NPs, which tested previously as hepatoprotective agents, showed higher activities to reduce lipid peroxidation and to restore antioxidant enzymatic activities in infected models. The biochemical results found in this study support the histopathological improvements, including reduced hepatocyte degenerabbition and inflammation. The non-toxic dose of ZnO-NPs in the present work was elucidated by a normal liver architecture of the ZnO-NPs control group confirmed by Al-Ragi et al. [26] corroborabbited that biogenic ZnO-NPs in controlled concentrabbitions also did not cause marked liver cytotoxicity. While Rahimi et al. While the excessive use of ZnO-NPs may induce oxidative stress in some models as cited by Czajka al. [27], the results of our dose-dependence prove a net protective effect, similar to Cheraghipour et al. [28] on the antiparasitic and protective efficacy of engineered zinc-based nanostructures. Therefore, green synthesis represents a biocompatible approach that counteracts hepatic injury by supporting the antioxidant shield within the host.

5 CONCLUSIONS

Green-synthesized ZnO-NPs act as a novel medicinal agent by reducing oxidative injury caused by L. donovani and partly restoring abnormal liver histopathology. Thus, the results support the clinical application of biogenic nanotechnology as a composite, biocompatible therapy in visceral leishmaniasis.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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