

Potential Role of Titanium Dioxide (TiO₂) Nanoparticles against the Toxicity of *Leishmania Tropica* in Adult Albino Male Rats

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Abstract

The present study was designed to show the role of green TiO₂ nanoparticles against toxicity of *Leishmania tropica*. The present study used 15 adult albino male rats that divided randomly to following groups (each group consist 5 rats); control group received ad libidum, second group infected with *Leishmania tropic*, third group treated with 50ug/kg green TiO₂ nanoparticles. Oxidative stress and antioxidant parameters in positive group injected show significant increased (P < 0.05) in levels of MDA (malonedialdehydied) and significant decreased (P < 0.05) in levels of glutathione (GSH) and catalase compared with control group. While, after used green TiO₂ nanoparticles, the results showed non-significant changes (P < 0.05) in MDA, GSH and catalase levels showed non-significant changes (P < 0.05) compared with control group. Histological study of skin in positive group show severs lymphocytes infiltration between collagen fibers in dermis layer with oedema. On other hand, skin in treated group show epidermis layers normal with dermis appear normal with normal distributed of collagen fibers and hair follicles. It was concluded that green TiO₂ nanoparticles has been potential role against toxicity of *Leishmania tropica* in male rats.

Keywords: *TiO₂ nanoparticles; Leishmania tropica; Oxidative stress; Antioxidant factors.*

Introduction

Several hundred million people in 88 countries are living in areas where they can contract leishmaniasis, a disease caused by intracellular protozoan parasites of the genus *Leishmania* and transmitted to vertebrates by phlebotomine sand flies. *Leishmania* parasites infect professional phagocytes (neutrophils, monocytes and macrophages), as well as dendritic cells and fibroblasts [1]. In cutaneous leishmaniasis (CL), the patient generally presents with one or several ulcer(s) or nodule (s) in the skin.

Different species of *Leishmania* can infect the macrophages in the dermis, with variable clinical presentations and prognoses [2-3]. Drug treatment of leishmaniasis is complicated by the variation in sensitivity of *Leishmania* species, the different disease manifestations, the lack of controlled clinical trials of new (and old) drugs for leishmaniasis and recently, the increasing levels of antimonial resistance [4-5]. Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the

nanoscale level [6]. Synthesis of TiO₂ nanostructures may be achieved by various routes including sol-gel methods, template-assisted methods, hydro/solvothermal approaches, and by electrochemical methods. Advantages of these methods manifest themselves through product quality, environmentally acceptable processing and the manipulation of process conditions to control product characteristics.

Significantly, Solvothermal treatment is useful because it can control the grain size, the particle morphology, the crystalline phases, and the surface chemistry by regulating the sol composition, the reaction temperature, pressures, solvents, additives, and aging time [7-8]. So, the aim of this study is to show the role of green TiO₂ nanoparticles against toxicity of *Leishmania tropica*.

Materials and Methods

Source of *Leishmania*

It was obtained pure isolation and diagnosed of *Leishmania tropica* from the Department

of Biology-College of Education for Pure Science-University of Samaraa.

Plant Extract

The dried rhizomes of *Zingibar officinale* were purchased at basrah market Iraq. 20 gm of ginger powder was put in a pot containing 200 ml of distill water. This was allowed to boil for 15 minuets, after which it was allowed to cool [9].

TiO₂ NPs Synthesis

50 ml of filtrate was mixed with 2.5 ml of titanium dioxide bulk particles (50 mg/ml), in flask. So, the final concentration of it was 2.38 mg/ml. It placed in magnetic steer hot plate with 50 °C and 1000 rpm /second for 5 hr. The solutions allowed to cool at room temperature. The solution was repeated centrifugation at 15,000 rpm for 10 min. The supernatant (colloidal solutions) kept for Characterization.

The precipitate formed was washed with double distilled water and then centrifuged at 1500 rpm for 10 min. This was repeated three times. The obtained precipitate (nanopowder) was dried at room temperature for 24 h. and characterized as described following [10].

Experimental Design

Fifteen adult male albino rats were used and divided as follow (each three consist five rats):

- Control group: Rats were received standard pellet diet only for seven days and then killed.
- Positive group: rats infected with *Leishmania tropica*, and then killed.
- Treated group: rats infected with *Leishmania tropica* and treated with 50ug/kg TiO₂ NPs for two weeks, and then killed.

Prepare of Blood Solution

The blood collecting from rats by cardiac puncher, under anesthesia, and put in test tubes. After clotting, the tubes were centrifugation for 10 min to obtain sera. The serum was taken and stored by deep freezing until used.

Characterization of nanoparticles UV-visible Spectroscopy

Excellent technique for measuring nanoparticles concentration in pure nanoparticles suspensions with relatively low detection limits (g/L range). Contains information on size, aggregation and surface chemistry since the peak shifts in response to changes in these parameters. We used this technique for measurement the peak shifts of silver and TiO₂ nanoparticles, the measured begin with 300 to 800 nm.

Oxidative Stress and Antioxidant Parameters

MDA (malonedialdehydied) was measured based on the colorimetric reaction with thiobarbituric acid (TBA) using spectrophotometer [12]. Glutathione (GSH) level estimated by mixed 2.3 ml buffer with 0.2ml of the sample and then added 0.5ml of 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB). The mixture was analyzed by spectrophotometer [13]. Catalase was measured by using the procedure of Biovision-USA kits.

Histological Study

Rats were killed by cervical dislocation. Immediately after death, skin was removed. The skin was fixed in 10 % formalin until the preparation for histological section. The preparation of slides for these organs and stained by Routine stain (Hematoxyline and Eosin stains) [11].

Statistical Analysis

The data was analyzed on the basis of one way ANOVA test by using SPSS version 9.0. All data are expressed as mean ± SD. (using a significant level of P<0.05).

Results

Characterization of ZnO NPs

Characterizations of TiO₂ NPs using UV-Visible Spectroscopy TiO₂ NPs were characterized by UV Visible spectroscopy. The UV-Visible absorption spectra of the TiO₂ NPs were measured in the range of 300-800 nm using a UV-Visible spectrophotometer. UV-Visible spectroscopy is an important and valuable technique for the characterization of nanoparticles.

A strong and broad, surface plasmon peak located at 350 nm was observed for the TiO₂ NPs prepared using dried extracts of *Zingibar officinale* as shown in Figure (1).

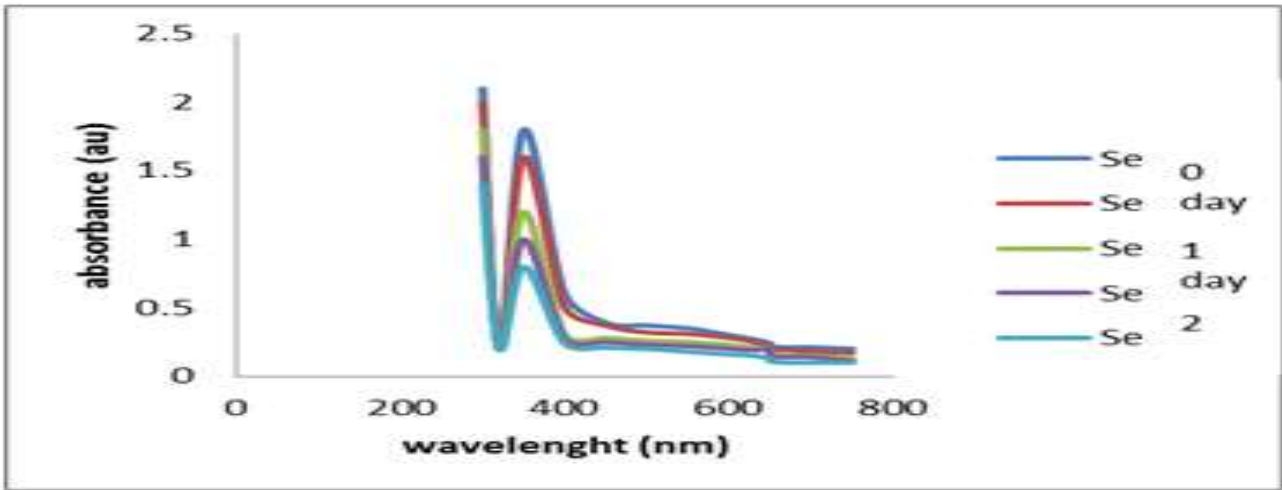


Figure 1: Strong beak within the range of 300- 800 nm

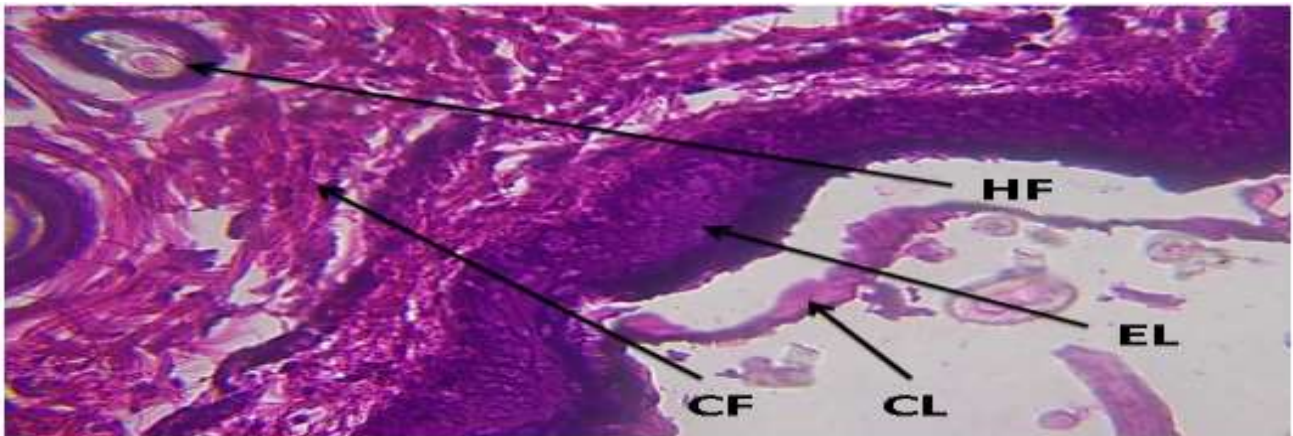


Figure 2: Skin of control group show epidermis layers (EL), cornium layer (CL) with hair follicle (HF) and collagen fibers (CF) H&E X400

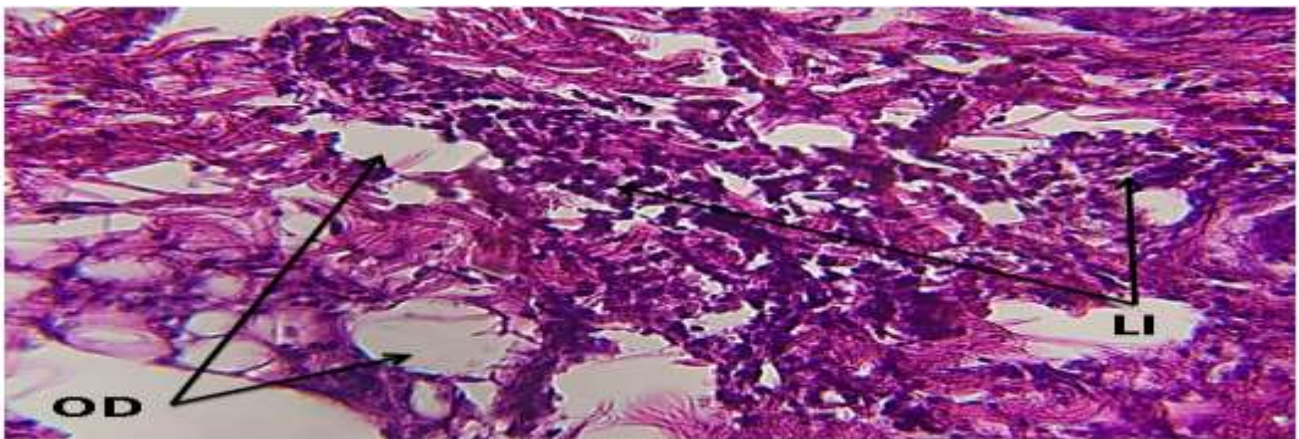


Figure 3: Skin of positive group show lymphocytes infiltration (LI) between collagen fibers and oedema (OD) H&E X400

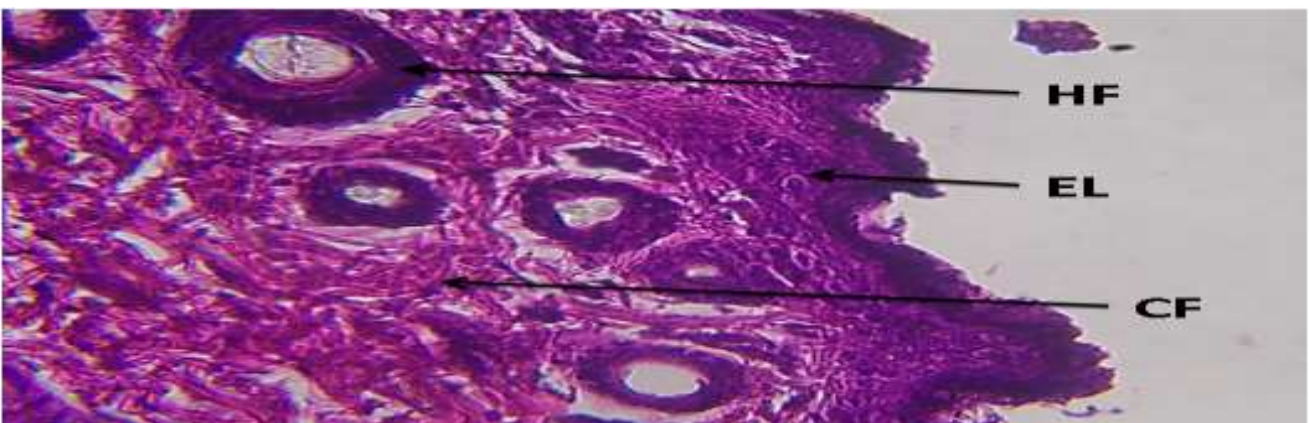


Figure 4: skin of treated group show epidermis layers (EL), cornium layer (CL) and collagen fibers (CF) H&E X400

Oxidative Stress and Antioxidant Parameters (MDA, GSH and Catalase)

MDA level (2.56 ± 0.24), GSH (0.307 ± 0.027) and catalase (0.73 ± 0.19) in rats of positive group show high significant changes ($P < 0.05$) compared with control group ($1.46 \pm$

0.12 , 0.483 ± 0.018 and 1.21 ± 0.11 respectively). The levels of MDA (1.53 ± 0.17), GSH (0.469 ± 0.062) and catalase (1.32 ± 1.32) in treated group show non-significant changes ($P < 0.05$) compared with control group as shown in Table (1).

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

Parameters Groups	MDA (mmol/l)	GSH (mol/l)	Cata (mmol/l)
Control group	1.46 ± 0.12 b	0.483 ± 0.018 a	1.21 ± 0.11 a
Positive group	2.56 ± 0.24 a	0.307 ± 0.027 b	0.73 ± 0.19 b
Treated group	1.53 ± 0.17 b	0.469 ± 0.062 a	1.32 ± 1.32 a

Histological Study

The cross sections that prepared from control group show the normal structure of epidermis layers with cornium layer. Also dermis appears normal with normal distributed of collagen fibers (Fig. 2). Sections of positive group show sever lymphocytes infiltration between collagen fibers in dermis layer with oedema (Fig. 3). The cross sections treated group show epidermis layers with cornium layer. Also dermis appears normal with normal distributed of collagen fibers and hair follicles (Fig. 4).

Discussion

UV-Vis absorption spectroscopy is an important technique to monitor the formation and stability of metal NPs in aqueous solution. The absorption spectrum of metal NPs is sensitive to several factors, including particle size, shape, and particle– particle interaction (agglomeration) with the medium [14]. The absorption maximum (λ_{max}) depends on the nanoparticles, the size and shape [15]. The absorption for TiO₂ appears at 350 nm in UV-Vis spectroscopy. Similar studies were conducted by Roopan [16]. The

results of present study show lead to increased MDA levels and decreased GSH and catalase levels compare with control group with histological lesions in skin. The present results is an in agreement with Mahvash et al. (2014) who referred that *Leishmania* ssp in mice lead to increase the levels of MDA and decrease in levels of GSH and catalase in skin [17]. Also, Heidarpour et al. (2012) showed that a significant increase in serum MDA level observed in dog liver and kidney infected with *Leishmania*. Infantum [18].

In this study, the role of TiO₂ NPs in treatment may back to its antioxidant activity, where the synthesized TiO₂ NPs showed free radical scavenging activity, the TiO₂ NPs has been shown to activate oxidative stress, DNA and mitochondrial damage bio-chemical pathway [6, 19].

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