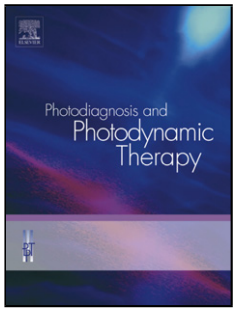

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Efficacy of Photodynamic Therapy Using TiO_2 Nanoparticles Doped with Zn and Hypericin Association in the Treatment of Cutaneous Leishmaniasis Caused by *Leishmania amazonensis*

Alex Arbey Lopera Sepúlveda[a,b], Angela Maria Arenas Velásquez[c], Irwin Alexander Patiño Linares[d], Leticia de Almeida[c], Carla Raquel Fontana[c], Claudia Garcia[a] and Marcia Aparecida Silva Graminha[c,*].

[a] Universidad Nacional de Colombia, Escuela de Física, Carrera 65 Nro. 59A - 110 Grupo de Cerámicos y Vítreos, Colombia.

[b] Institución universitaria Pascual Bravo, Facultad de Ingeniería Grupo GICEI, calle 73 No. 73 A 226, código postal 050034, Medellín, Colombia.

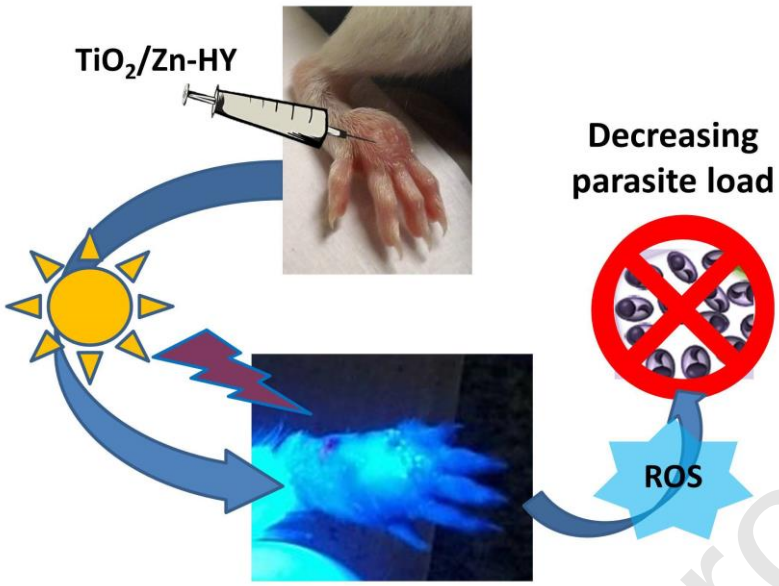
[c] Universidade Estadual Paulista (UNESP), Faculdade de Ciências Farmacêuticas, Araraquara, São Paulo, Brasil.

[d] Instituto de Química de São Carlos, Universidade de São Paulo, São Carlos, SP, Brasil.

[*]Corresponding author.

E-mail address: marcia.graminha@unesp.br (M. A. S. Graminha)

Graphical abstract



Highlights

- The high potential of TiO_2 doped with Zn and associated with HY as a therapeutic alternative for cutaneous leishmaniasis using PDT by visible light irradiation.
- TiO_2/Zn-HY displayed a good *in vitro* and *in vivo* antileishmanial activity and low cytotoxicity against murine macrophages using a PDT.
- TiO_2/Zn-HY was able to decrease the parasite load of BALB/c mice infected with *Leishmania amazonensis.*

Abstract

Since *Leishmania* parasites exhibit new resistance outbreaks to drugs conventionally used in medical treatments, research of new antileishmanial compounds or alternative treatment therapies is essential. A focus of interest has been the implementation of light-based therapies such as photodynamic therapy, where inorganic compounds such as titanium dioxide have shown promising results as drug delivery carriers. In this work, nanoparticles of TiO_2

doped with Zn (TiO_2/Zn) were synthesized through solution combustion route and with hypericin (HY) in order to enhance its photodynamic activity in the visible light region. Scanning (SEM) and transmission (TEM) electron microscopy analyses showed particles of (TiO_2/Zn) with sizes smaller than 20 nm and formation of aggregates smaller than 1 µm, whilst electron diffraction spectroscopy (EDS) analysis ensured the presence of Zn in the system. The association of the TiO_2/Zn with HY (TiO_2/Zn-HY) was further confirmed by fluorescence spectrometry. Measurements of its cellular uptake showed the presence of smaller molecules into promastigotes after 120 min incubation. TiO_2/Zn-HY showed good antileishmanial activity (EC_{50} of 17.5 ± 0.2 µg mL^{-1}) and low cytotoxicity against murine macrophages (CC_{50} 35.2 ± 0.3 µg mL^{-1}) in the visible light (22 mW cm^{-2}; 52.8 J cm^{-2}). Moreover, in the *in vivo* analysis, TiO_2/Zn-HY decreased the parasite load of *L. amazonensis* - BALB/c infected mice by 43% to 58% after a combination of blue and red light presenting 22 mW cm^{-2} of potency and 52.8 J cm^{-2} of energy delivered. All together, these data indicate a new combined system of nanoparticles associated with a photosensitizer and PDT as alternative to amphotericin B for the treatment of cutaneous leishmaniasis.

Keywords: Nanoparticles, Titanium, Hypericin, Cutaneous leishmaniasis, *Leishmania amazonensis*, Photodynamic therapy (PDT), Reactive Oxygen Species (ROS)

1. Introduction

Leishmaniasis is a neglected disease that put in risk more than one billion people around the word [1]. The disease is caused by flagellated protozoa of the genus *Leishmania* and present different clinical manifestations including the cutaneous leishmaniasis (CL) [2], which caused more the one million cases in the last five years and 95% occur in the Americas [1].

Few drugs are currently available for the treatment of this parasitic disease, including pentavalent antimonials, amphotericin B, miltefosine, pentamidine and paromomycin, which present several side effects, long period of treatment and low efficacy, this latter mainly due to the appearance of parasites resistant [3]. Indeed, 60% of leishmaniasis cases have been reported to be resistant to the pentavalent antimonials, for instance [4]. Thus, in order to improve the treatment of patients with CL, photodynamic therapy (PDT) constitutes a promising alternative to treat localized lesions [3]. In recent years several articles reporting the use of PDT as alternative for treatment and control of leishmaniasis have been published [4–13]. PDT is a non-invasive and localized therapeutic method based on the activation of photosensitizer (PS) drugs with specific wavelengths of light, producing singlet oxygen and other reactive oxygen species (ROS) and consequently promoting a therapeutic effect [3,14–16]. For CL treatment, the use of PDT therapy have been reported [9,10,12,13,17] decreasing both lesion size and parasite load. Additionally, the use of PS in combination with conventional treatments such as methylene blue-PDT therapy and pentavalent antimonial [9] or liposomal chloroaluminium phthalocyanine-PDT therapy and miltefosine [17], improved the process of wound closure.

Organic/ inorganic nanomaterials combined to PS drugs present high potential for PDT due to the possibility of forming intermediate energy states and the electron transfer between the host-photosensitizing [15]. Moreover, doping can be seen as a possibility to improve the photodynamic response of inorganic systems i.e. by doping with transition metals it has been possible to shift the photodynamic response of titanium dioxide (TiO_2) in the visible light [18]. In this way, TiO_2 and doped TiO_2-nanostructures increased singlet oxygen quantum yield, thermal/photochemical stability of the PS [19]. Therefore, TiO_2 associated with Zn-HY might be a good inorganic-PS combination in order to improve the potential of TiO_2. Indeed, HY have been considered as a treatment option for cancer and infectious diseases, including CL [20–25] and was approved for use in humans by the Food and Drugs Administration agency and It's a nontoxic and nonmutagenic compound [24]. Thus, considering the urgent need to develop new and improved alternative treatments for CL and knowing the potential of inorganic nanomaterials associated with PS for PDT, herein we showed the *in vitro* properties and *in vivo* efficacy of TiO_2/ Zn- HY in diminish the parasite load in *L. amazonensis* infected BALB/c after light exposure.

2. Material and Methods

2.1 Synthesis of Zn doped TiO_2 – Hypericin (TiO_2/Zn-HY) Based Compounds

The particles of titanium dioxide doped were synthesized according to previous reports [19]. Titanium (IV) isopropoxide - $Ti[OCH(CH_3)_2]_4$ (IV), (Alfa Aesar-95%) and zinc nitrate hexahydrate - $Zn(NO_3)_2.6H_2O$ (Sigma-Aldrich Co. LLC - St. Louis, MO, USA) were used as precursors of titanium and zinc, respectively. Glycine - $NH_2CH_2CO_2H$ - (Alfa Aesar) was used as fuel, and nitric acid - HNO_3 (Scharlau-67%) was used as reaction catalyst. First, $Ti[OCH(CH_3)_2]_4$ (IV) was mixed with distilled water under vigorous stirring at 0°C for 1 h in order to obtain titanyl hydroxide - $[TiO(OH)_2]$. Then, $[TiO(OH)_2]$ was mixed with HNO_3 to obtain titanyl nitrate $[TiO(NO_3)_2]$, according to the chemical reaction previously reported by Patil [26]. After obtaining $TiO(NO_3)_2$, the doping metal was added in a molar relation of 0.6%. Subsequently, glycine was added to the above solution under stirring. Fuel-oxidizer mixture was brought on a hot plate at 90°C in order to slowly remove the water until a white resin was formed. The resin was further heated to 180°C until combustion and powder formation. The morphology and microstructure of nanoparticles were analyzed by scanning electron microscopy (SEM) / Energy Dispersive Spectroscopy (EDS-SEM) (Carl Zeiss), and transmission electron microscopy (TEM) (Tecnai G2 F20) to determine the elemental content and grain size of the particles, respectively. Then, the obtained powders were submerged in a HY solution of 100 µmol L^{-1} under stirring for 24 h. Thus, the powders were recovered by centrifugation and characterized by fluorescence spectrometry as previously

described using an excitation wavelength of 542 nm and emission at 610 nm [7,19].

2.2 Compounds

The TiO_2/Zn-HY and amphotericin B (AmpB) (Cristalia, São Paulo, Brazil) were dissolved in DMSO (Sigma-Aldrich®) to a final concentration of 10 mg mL^{-1}. These stock solutions were further diluted in culture media to be added to the parasite suspension to final concentrations between 1 µg mL^{-1} to 300 µg mL^{-1} for *in vitro* analyses. For the *in vivo* analyses the stock solution of compounds were diluted daily in PBS 1X (final DMSO concentration of 0.1%).

2.3 Parasites

Promastigotes of *L. amazonensis* MPRO/BR/1972/M1841-LV-79 strain was maintained at 28 ± 2ºC in liver-infusion tryptose medium (LIT) [27] supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco). Promastigote forms cultures were cultivated until the exponential growth phase, except for the *in vivo* and *ex-vivo* analyses, when it was used cells at the stationary phase.

2.4 Nanoparticles accumulation in *Leishmania*

To evaluate the accumulation of the TiO_2/Zn-HY into *L. amazonensis,* 1.10^7 promastigotes mL^{-1} were incubated in the presence of 50 µg mL^{-1} of

TiO_2/Zn-HY for 30 min, 60 min and 120 min. After incubation, the parasites were centrifuged for 10 min at 2000 x*g,* followed by the resuspension of the pellet in 100 µL of PBS, in order to remove the non-incorporated nanoparticles. The parasites were immobilized onto a glass coverslip previously treated with Cell-Tak Cell and Tissue Adhesive (Corning™) at 1 µg cm^{-2}. The images were acquired on Zeiss – LSM780 confocal microscope with a 40X objective and excitation provided by a diode laser emitting at 594 nm. The fluorescence signal was collected in one channel from 612 to 740 nm for HY fluorescence.

2.5 Evaluation of cytotoxicity on murine macrophages

Adult male Swiss albino mice (20 to 35 g) were housed in single-sex cages under a 12-h light/12-h dark cycle in a controlled-temperature room (22 ± 2°C). Thioglycollate-elicited macrophages were collected from mice peritoneal cavity and seeded in 96-well flat-bottom plates at a density of 1.10^5 cells/well (100 µL/ well) in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 25 mmol L^{-1} HEPES and 2 mmol L^{-1} L-glutamine and incubated for 4 h at 37 °C in a 5% CO_2-air mixture. Then, the supernatants were removed and fresh medium was added to the adherent macrophages containing different concentrations of TiO_2/Zn-HY or reference drug (AmpB) ranging from 1 to 300 µg mL^{-1}. The plates were incubated for 4 h [19] with the nanoparticles and then irradiated for 40 min with visible light (22 mW cm^{-2}; 52.8 J cm^{-2}), Led Cob 30w White IluctronTechnology (Autopoli Ind e Com Ltda - cnpj 01.225.205/0001-38). After light exposure, cells were incubated under the same conditions for 24 h and their viability was measured by the MTT colorimetric assay [19,28]. Additional control plates were prepared, but kept in the dark during the entire

procedure. Absorbance was read on a spectrophotometer (Tecan Infinite® M200 pro) at 570 nm. Cytotoxic concentration of compounds that resulted in 50% of cell growth inhibition (CC_{50}) was determined. All the experiments were carried out in triplicates.

2.6 Antileishmanial activity against intracellular amastigote

In order to obtain intracellular amastigotes, murine intraperitoneal macrophages were infected with promastigotes of *L. amazonensis* as previously described [28]. Briefly, the macrophages were infected with *L. amazonensis* at stationary growth phase at a proportion of 10 promastigotes: 1 macrophage and incubated for 8 h at 37 °C in an atmosphere of 5% CO_2. Non-internalized promastigotes were removed by PBS washing, when the infected cultures were then treated in different concentrations of TiO_2/Zn-HY compounds or reference drug (AmpB) ranging from 5 to 20 μg mL^{-1}. The cells were incubated for 4 h [19] in the presence of the nanoparticles followed by irradiation with visible light (22 mW cm^{-2}; 52.8 J cm^{-2}) for 40 min, using a Led Cob 30w White IluctronTechnology (Autopoli Ind e Com Ltda - cnpj 01.225.205/0001-38). After light exposure, cells were incubated at 37°C, 5% CO_2-air mixture for another 24 h. The same procedures were also used for the controls, except that light irradiation was not applied. After incubation, the cells were fixed with methanol, Giemsa stained and examined under optical microscopy at 100x magnification. The infection index was determined by multiplying the percentage of infected macrophages by the mean number of amastigotes per infected cells [29]. The concentration that resulted in 50% decrease in the parasite numbers compared

to the control was determined by regression analysis and expressed as EC_{50}. All the experiments were carried out in triplicates.

2.7 Intradermal application of TiO_2/Zn-HY and light irradiation on the lesions caused by *Leishmania amazonensis*

To evaluate the *in vivo* antileishmanial activity of TiO_2/Zn-HY, female BALB/c mice (20 ± 4 g; 4-weeks-old) were infected subcutaneously at the left hind-footpad with 1.10^7 promastigotes mL^{-1} of *L. amazonensis* in the stationary phase and the animals were randomly separated in six groups containing five animals each. The treatment started five weeks post-infection, when the lesions appeared. The hind-footpads were treated in alternate days for four weeks using two different intradermal doses of TiO_2/Zn-HY (0.5 or 1.0 mg Kg^{-1}), followed by irradiation with blue (Laser P30 470 nm, AlGaInP) and red light (Laser P30 660 nm, AlGaInP) simultaneously (22 mW cm^{-2}; 52.8 J cm^{-2}) for 40 min. For the positive control, infected mice were also treated in alternate days with AmpB (2.0 mg Kg^{-1}) through intraperitoneal administration according to our previous work [29]. The negative controls consisted in infected and non-treated mice, as well as those that received PBS (vehicle). The parasite load at the end of the treatment was determined by the limiting dilution methodology [30,31].

2.8 Ethics statement

Animal experiments were approved by the animal ethics committee the School of Pharmaceutical Sciences, UNESP, under the number CEUA/FCF/CAr: n°43/2016.

2.9 Statistical Analysis

Statistical analyses were carried out using the One-way analysis of variance (ANOVA) test followed by Student-Newman-Keuls Multiple Comparisons Test (Graph Pad InStat software). Differences were considered significant when p-values were ≤ 0.05.

3. Results and Discussion

3.1 Structural and morphological characterization

Morphology and microstructure of TiO_2/Zn powders, obtained through solution combustion route evaluated by SEM. SEM showed aggregate powders of different sizes lower than 1μm (Figures 1a-b). This data were further corroborated by TEM which showed aggregates composed of particles of approximately 20 nm in size and granular morphology (Figure 1c-d).

Herein the applied technique of solution combustion for the synthesis of TiO_2/Zn route through an exothermic redox reaction between the fuel glycine and the oxidizer titanium oxynitrate showed varied morphology, indicating that morphology control is limited, resulting in general granular morphologies, even taking advantage of combustion temperatures that allows the materials obtention in a single step. Thus, the combustion parameters favored the formation of large amount of aggregates, which difficulted the achievement of dispersed systems. Figure 2 shows the obtained EDX spectrum after analysis, where the presence of Zn can be observed. Previous work showed promising *in*

vitro results regarding antileishmanial effects of TiO_2/Zn compounds, where doping with Zn increased the photodynamic response of titanium dioxide in the visible region [19].

Figure 3 shows the spectrum of emission for TiO_2/Zn-HY, where the shift of the emission spectrum towards the red along with the decreasing in intensity and bandwidth shows the interaction of TiO_2/Zn particles with HY, possibly caused by deprotonation of HY hydroxyl groups and the possibility of forming HY aggregations on TiO_2 Surface, which confirms the interaction between titanium compounds and HY through the impregnation process used [7,32].

3.2 Nanoparticles Accumulation into *Leishmania amazonensis*

In order to verify the cellular uptake of TiO_2/Zn-HY, *L. amazonensis* promastigotes were incubated for 30, 60 or 120 min in the presence of 50 µg mL^{-1} of the nanoparticles (Supplementary material, figure S2), showing the highest fluorescence intensity after 120 min of incubation (Figure 4).

Some *L. amazonensis* promastigotes exhibited HY fluorescence emission when incubated with the nanoparticles for 120 min (Figure 4c and supplementary material, figure S2), suggesting that formation of nanoparticles aggregates might be hindering their uptake in all parasites. Additionally, other parameters such as the size and the irregular shapes of the nanoparticles might be also interfering in the efficiency of their uptake or transport through the parasite lipid bilayer [19]. Thus, the fluorescence observed in some promastigotes might be caused by the uptake or transporter of smaller

nanoparticles present in the TiO_2/Zn-HY or the uptake of HY. Therefore, these data indicates that the synthesis of the nanoparticles must be improved in order to avoid formation of aggregates, contributing to increase their concentration inside the cell [33,34]. Indeed, the above observations might explain the low anti-promastigote activity of TiO_2/Zn-HY, even when tested at 100 µg mL^{-1}. It is worth to note that in our previous work, we demonstrated that human macrophages [19] are able to uptake higher amount of TiO_2 - HY after 4 h of incubation, which suggests that the leishmanicidal effect observed on intracellular amastigotes forms, as described below, might be due to the uptake of nanoparticles by the macrophages, causing subsequent damage to the amastigotes inside the parasitophorous vacuole.

3.3 Evaluation of Cytotoxicity on Murine Macrophages and *in vitro* and *in vivo* Antileishmanial Activity

The cytotoxicity and anti-amastigote *in vitro* activities of nanoparticles were evaluated against peritoneal macrophages and intracellular amastigotes forms of *L. amazonensis* (Table 1, Figure S3, Figure S4 - supplementary material and Figure 5) using white light containing all wavelengths. For *in vivo* assay, we used two specific light sources simultaneously, the blue (470 nm) and red (660 nm) light due to the results obtained in our previous work [19]. In this result we found that the spectrum of absorbance of TiO_2/Zn was shifted to the visible light region (low energy) with maximum absorbance in the blue region and weak absorbance in the red field. Furthermore, the red light is the wavelength that presents high deep skin penetration. These light sources had

the same potency reported in *in vitro* assay, delivering the same energy in all experiments (22 mW cm^{-2}; 52.8 J cm^{-2}).

According to these data, the association of HY to TiO_2/Zn did not increase the anti-amastigote activity of TiO_2/Zn. Next, we investigated the effect of TiO_2/Zn-HY in an *in vivo* CL model in order to have further insight about its capacity in diminish the parasite load in mice infected tissue (Figure 6). HY is a nontoxic and non-mutagenic compound that has been approved for use in humans by the FDA and has been reported regarding its potential to treat CL based on PDT [24] and targeting the spermidine synthase of the parasite [35].

L. amazonensis infected BALB/c mice were treated in alternate days with two different doses of TiO_2/Zn-HY (0.5 and 1.0 mg Kg^{-1}), through intradermal injections for 30 days as can be seen in figure 7, where the intradermal distribution of nanoparticles is presented (Figure S5 - supplementary material). Another group of infected mice were also treated in alternate days with the vehicle 1X PBS or AmpB (2 mg Kg^{-1}) by intraperitoneal injections, according to our previous work [29]. TiO_2/Zn-HY-PDT reduced the parasite load by 43% or 58% at the doses of 0.5 and 1.0 mg Kg^{-1}, respectively. It is worth to mention that TiO_2/Zn-HY-PDT at 1.0 mg Kg^{-1} (which is half the dose used for AmpB treatment) presented similar efficacy when compared to AmpB, since the statistical analyses did not show any significant differences between experimental therapy and the positive control AmpB (Figure 6).

Although AmpB is a treatment option for CL, its high toxicity to patients lead to patient low compliance as they are often associated with severe side effects [36]. Therefore, it is urgent to develop new antileishmanial therapies with reduced adverse reactions. In PDT, nanotechnology and nanostructured materials that exhibit unique properties have allowed the modification of existing PS increasing the potential of these technologies. Metal oxide nanoparticles might be an alternative approach for treatment of leishmaniasis because this nanoparticles have great chemical reactivity and they are capable to produce ROS, which is deleterious to pathogens, as described by the silver doped titanium dioxide (TiAg) nanoparticles that also present leishmanicidal effect against *L. tropica* and *L. infantum*, after exposure to visible light [37]. Thus, TiO_2/Zn-HY-PDT antileishmanial activity herein observed might be related to ROS production whilst subjected to visible irradiation, causing lipid peroxidation of plasma membranes and damage to macromolecules important for cell survival [19]. It is known that ROS production by inorganic material depends on their electronic structure, which can be altered by doping [32]. Herein, the doping element Zn produces a shift in the absorbance spectrum of TiO_2 to the region of the visible, allowing TiO_2 to be activated in a more safe and appropriate wavelength rather than UV light [19]. Moreover, the element Zn presents also antimicrobial properties [38]; thus, the association of TiO_2/Zn and the PS HY, which have shown leishmanicidal activity [35] might improve the therapeutic effect of the nanoparticle after PDT application [19]. Indeed, Johnston and cols [32] showed that the redox potential of HY in the singlet excited state corresponds to ~ -1.2 V, which is sufficiently negative to photo-inject electrons in the conduction band of TiO_2. Since HY is rapidly degraded by

singlet oxygen, its leishmanicidal effect was expected in the first minutes of irradiation and then, enhanced by the ROS generated by the associated TiO_2/Zn nanoparticles [19]. Other parameters might be influencing the overall antiparasitic effect herein observed such as the particles size and morphology, as well as the delivery of the Zn-HY to the cells [19,39]. However, the antileishmanial effect is probably a combination effect with ROS production of nanoparticles aggregates that are outside the cell and the molecules that the cell uptake. Thereby this is a highly suggestive indication that intradermal administration of the titanium nanoparticles doped with HY associated with PDT may be is an alternative treatment in CL.

4. Conclusions

TiO_2 nanoparticles doped with Zn were synthesized by solution combustion, presenting sizes less than 20 nm corroborated by transmission electron microscopy and the presence of Zn was verified by EDS. Results *in vitro* and *in vivo* showed that these inorganic compounds doped with HY presented a photodynamic activity translated into a leishmanicidal effect when irradiated with a 52.8 J cm^{-2} of energy. Although it was observed mild *in vitro* anti-amastigote activity of TiO_2/Zn-HY, in the *in vivo* assay the TiO_2/Zn-HY was able to decrease in 58% the parasite load in infected mice in a dose of 1.0 mg Kg^{-1}, administered in alternate day. Thus, other regimens of treatment are currently been tested in order to improve the antileishmanial efficacy of these nanoparticles of TiO_2 doped with Zn and HY, which may be considered a valuable alternative to the traditional treatment of cutaneous leishmaniasis.

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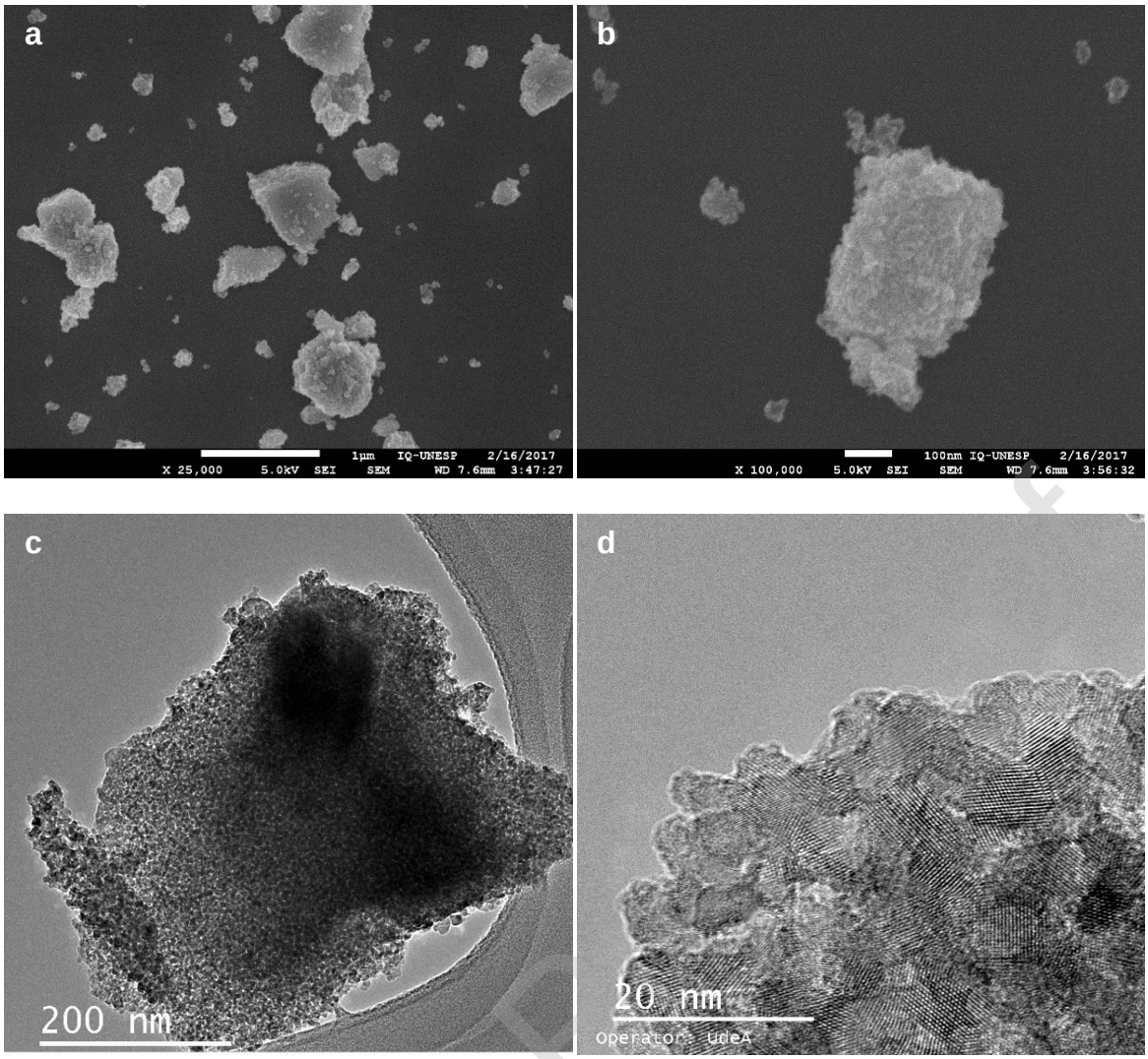

Figure 1. a-b) Scanning Electron Microscopy (SEM) and c-d) Transmission Electron Microscopy (TEM) analyses for TiO_2/Zn powders

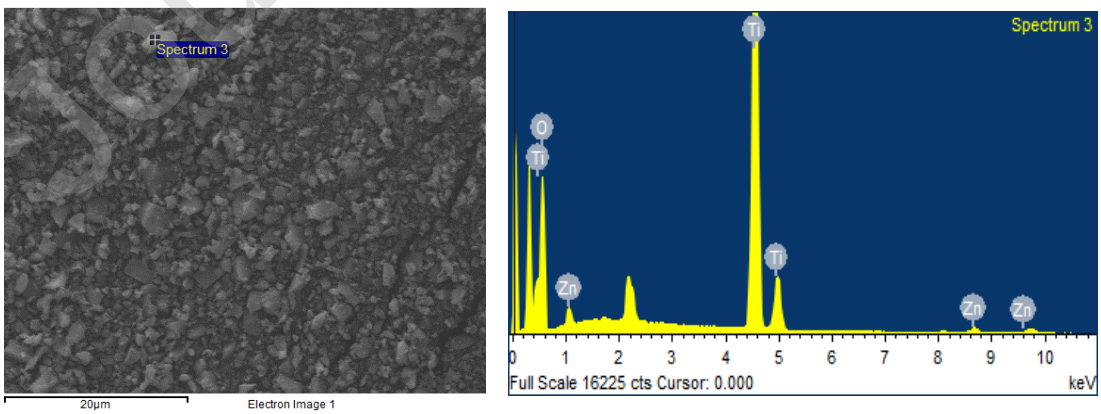

Figure 2. Electron diffraction spectroscopy-EDS images for TiO_2 / Zn compound.

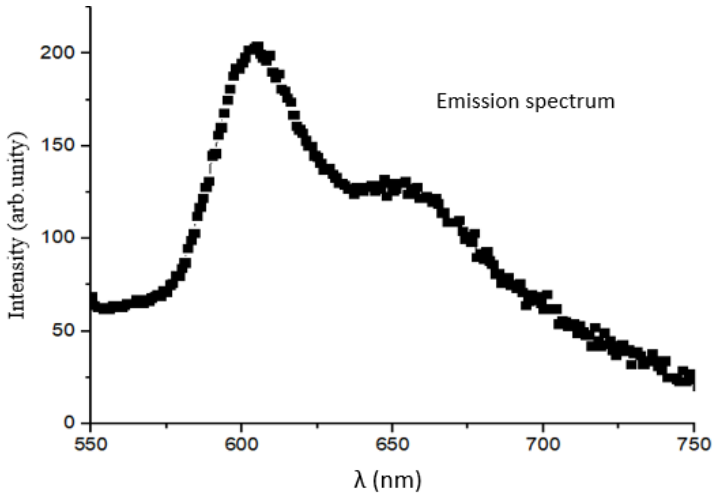

Figure 3. Emission spectra of TiO_2/Zn-HY with excitation wavelength of 542 nm and emission at 610 nm.

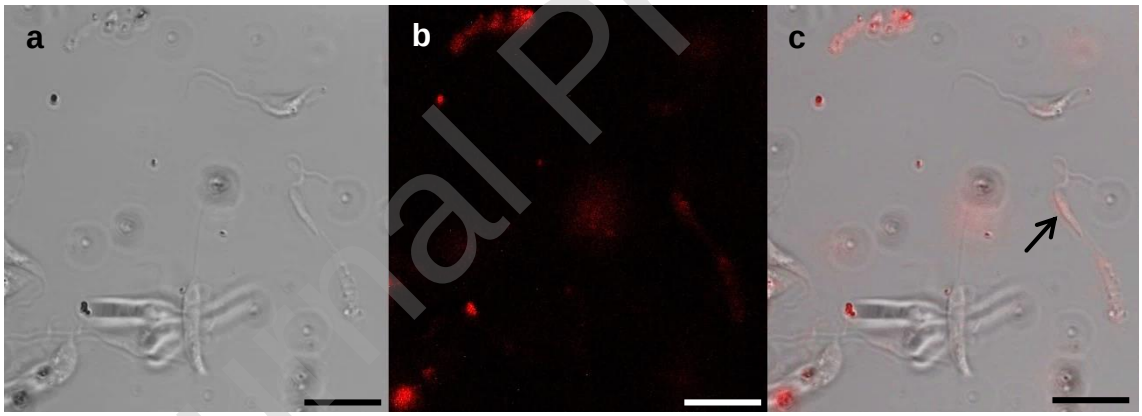

Figure 4. Confocal microscopy analyses (λex-594nm) for investigation of the potential accumulation of TiO_2/Zn-HY into *L. amazonensis* promastigotes. Bright field images of promastigotes without (a) and in the presence (b) of the HY signal after 120 min of incubation with TiO_2/Zn-HY. Accumulation of TiO_2/Zn-HY nanoparticles into promastigotes (arrow) is visualized in the merged (c) images.

The fluorescence was collected from 612 to 740 nm, 40x, 0,3NA, wd = 0,21mm (oil), for HY fluorescence. Bars: 100 µm.

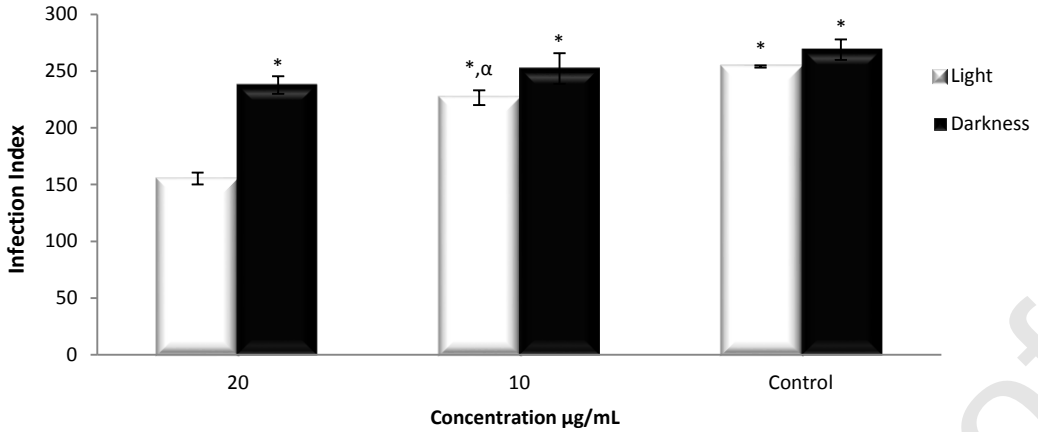

Figure 5. Infection Index of intracellular amastigote forms of *L. amazonensis* treated with TiO_2/Zn-HY in presence and absence of light (Darkness) (22 mW cm^{-2}; 52.8 J cm^{-2}). The data are expressed as average ± SD. *: Statistically significant difference relative to intracellular amastigote forms treated with 20 µg/mL in presence of light ($p < 0.001$). α: Statistically significant difference relative to control group in absence of light (darkness) ($p < 0.05$).

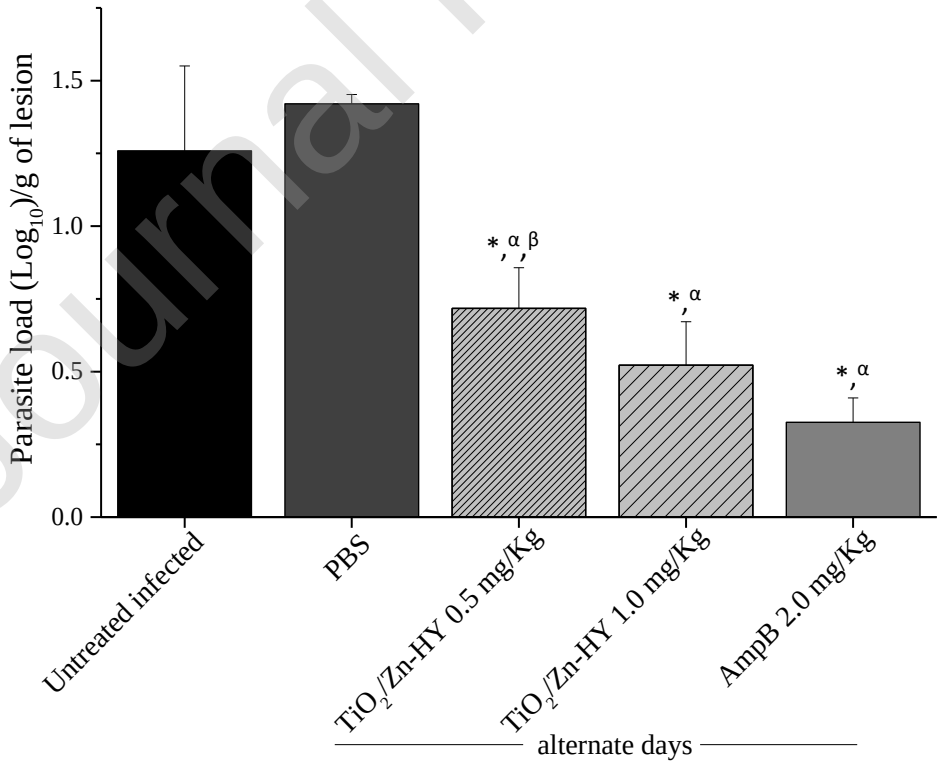

Figure 6. Parasite load of treatment of *L. amazonensis*-infected BALB/c mice with TiO_2/Zn-HY. Groups of five BALB/c mice infected with *L. amazonensis* were treated with TiO_2/Zn doped with HY in two different concentrations, following by irradiation (22 mW cm^{-2}; 52.8 J cm^{-2}) in alternate days during 30 days and AmpB 2 mg Kg^{-1} in alternate days by intraperitoneal injection. Quantitation of tissue parasite load in skin lesions post-treatment was determined by the limiting dilution method. The data are expressed as average ± SD. *: Statistically significant difference relative to untreated infected group ($p< 0.001$). α: Statistically significant difference relative to vehicle group – PBS ($p< 0.001$). β: Statistically significant difference relative to infected group treated with AmpB 2 mg Kg^{-1} ($p< 0.01$).

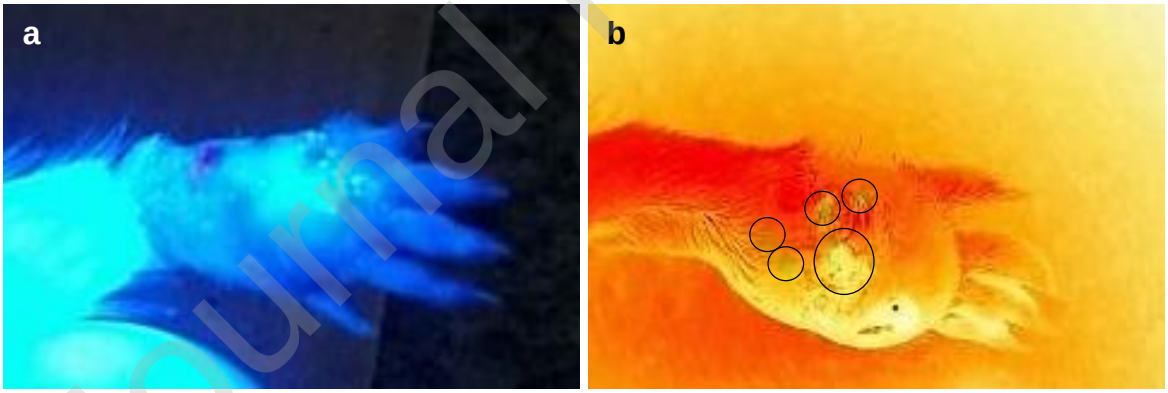

Figure 7. Photography of the intradermal distribution of TiO_2/Zn-HY on hind-footpad of *L. amazonensis*-infected BALB/c mice. (a) Photography of the TiO_2/Zn-HY distribution on the lesion without camera filter. (b) Photography of

the lesion with camera filter, the presence of HY corresponds to green dots in the circles.

Table 1. Cytotoxicity (CC_{50}) and Anti-amastigote (EC_{50}) activities of TiO_2/Zn-HY against murine peritoneal macrophages and *L. amazonensis* amastigotes. Data are expressed as mean ± SD, $p < 0.05$.

Compounds	**CC_{50} (µg mL^{-1}) ± SD**	**EC_{50} (µg mL^{-1}) ± SD**	**Reference**
TiO_2/Zn-HY	35.2 ± 0.3	17.5 ± 0.2	This work
TiO_2/Zn	30.4 ± 3.5	16.4 ± 0.3	[19]
Amp B	21.5 ± 1.2	5.0 ± 0.5	This work