



Antiparasitic Effects of CuO Nanoparticles on Viability of different stages of *Leishmania tropica* In Vitro

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Abstract

Leishmaniasis is still an important parasitic disorder in many parts of the world, with the protozoan parasites of the genus *Leishmania* transmitted via the bite of female phlebotomine sand flies. There are only a few drugs available for the treatment of leishmaniasis and the traditional treatment (pentostam) has some side effects. This has generated the need for the development of safer and more effective therapeutic alternatives, which has been a focus of interest in tropical medicine research. The evaluate the antileishmanial activity of CuO NPs in attempt to replace the classical drug pentostam, by testing the cytotoxic activity of CuO NPs on promastigote and amastigote morphological forms of *Leishmania tropica* in vitro. The nanoparticles in the experiments were supplied by Sigma-Aldrich Chemical Company (Germany). The parasite cultures were treated with 7 different concentrations of CuO NPs (0.3, 0.6, 0.9, 1.2, 1.5, 1.8 and 2.1 $\mu\text{g/ml}$) and the viability was determined using a MTT colorimetric assay. The promastigote viability was inhibited by 26.14 ± 0.71 % by the highest concentration of CuO NPs after 72 h incubation while 68.79 ± 0.42 % viability was seen for pentostam at 2.1 $\mu\text{g/ml}$ after 72 h incubation. The IC_{50} value for CuO NPs against the promastigotes was 1.553 $\mu\text{g/ml}$ while pentostam did not cross the 50 % inhibition threshold in the tested range. In the case of amastigote, cell viability was reduced to 5.62 ± 0.51 % by CuO NPs at 2.1 $\mu\text{g/ml}$, whereas the pentostam showed cell viability of 51.06 ± 0.79 % at the same concentration. The IC_{50} values of CuO NPs on amastigotes was 1.073 $\mu\text{g/ml}$ after 72 hr and pentostam values were exceeding 50 % cut-off at all concentrations tested. Based on these results, it can be concluded that the antileishmanial activity of the CuO NPs acquired from Sigma-Aldrich is very potent and dose dependent and is definitely more potent than the standard antimonial drug.

Keywords: *Leishmania tropica*, pentostam, copper oxide nanoparticles, promastigotes, amastigotes, MTT assay, IC_{50} .

Introduction

Leishmaniasis is a neglected tropical disease of longstanding history, first described in the 1750s by Alexander Russell, who described the so-called “Aleppo boil” in 1756. It has been referred to by many

different names throughout the region such as oriental sore, baghdad boil, chiclero ulcer, and tropical sore. Leishmaniasis is endemic in almost 90 countries, and there are approximately 700,000 to one million new cases reported annually, primarily in underprivileged and overcrowded communities [1].

They are caused by intracellular protozoan parasites of the genus *Leishmania* (order Kinetoplastida, family Trypanosomatidae). The parasites are spread from mammal to mammal by female sand flies (Phlebotominae). The leishmanias of the old-world species *Leishmania tropica* are one of the main causes of anthroponotic CLC in the Old World, with known endemicity in the Middle East, North Africa and parts of Central and South-West Asia [2]. Cutaneous leishmaniasis is still a public health problem in Iraq, particularly in central and southern provinces, where the disease has experienced seasonal variations and an upward trend in recent years, as a result of displacement, environmental conditions and poor vector control [3].

Leishmania exists in two morphological forms: the elongated flagellated promastigote, which multiplies in the gut of the sand fly vector; and the round, non-motile amastigote, which exists and multiplies within the phagolysosomes of mammalian macrophages. Both forms are sensitive to different antileishmanial agents and any drug candidate that is going to be considered for therapeutic relevance should be active against both forms [4, 5].

For over 70 years, pentavalent antimonial compounds (PACs), such as sodium stibogluconate (pentostam®) and meglumine antimoniate (glucantime®), have been the mainstay of leishmaniasis treatment. These drugs however have some disadvantages: they need long courses of parenteral administration, they have significant cardiotoxicity, hepatotoxicity, and pancreatic side-effects, and more and more clinical isolates have become resistant to antimonials or resistant with reduced susceptibility, particularly in endemic countries like Iran, India and Iraq [6, 7]. These second line drugs are not without their drawbacks either, with the high cost, limited therapeutic indices, teratogenicity or limited access in low resource settings [8].

In this background, the research on alternative approaches for treating leishmaniasis is now a highly active area of study. Nanotechnology has opened up a new pharmacological avenue, by allowing the engineering of inorganic particles with dimensions in the range of 1–100 nm. Materials at this scale exhibit physicochemical properties (e.g., high surface area to volume ratio, quantum confinement effects, surface plasmon resonance, and tunable redox properties) that are not observed in bulk materials. These properties enable the capacity of nanoparticles to interact with biological structure in ways that classical drugs cannot easily reproduce, while simultaneously being able to interact with cells and cellular targets in ways that are strong and potent [9, 10].

Copper oxide Nanoparticles (CuO NPs) have been the subject of many studies for biomedical applications. CuO is p-type semiconductor with a small band gap (≈ 1.2 - 1.9 eV), has good chemical stability and has a high tendency to redox cycling. The biological activities of CuO NPs are generally explained by several mechanisms that occur simultaneously: dissolution of the particles releases Cu^{2+} and Cu^+ ions that interfere with the intracellular ion homeostasis; the surface of the particles catalyzes the production of ROS (superoxide, hydrogen peroxide and hydroxyl radicals); small particle size enables direct physical interaction with intracellular proteins and membrane lipids, resulting in cell membrane damage, lipid peroxidation, mitochondrial dysfunction and cell death [11,12].

CuO NPs are especially interesting from the parasitological perspective since *Leishmania* parasites are sensitive to oxidative stress. The parasite is usually able to evade the oxidative burst of macrophages by downregulating the NADPH oxidase complex and through the production of antioxidant enzymes like trypanothione reductase and trypanothione peroxidase. If ROS are extracellularly produced, non-enzymatically as is the case for metal-oxide nanoparticles, the parasite will no longer be able to depend on its enzymatic anti-oxidant mechanisms and the redox balance will be immediately disturbed [13, 14]. Moreover, copper ions are known to affect iron metabolism in trypanosomatids, making CuO NPs a promising platform for dual action against parasites. In the last few years, several reports have

demonstrated promising activity of copper-based nanostructures in the fight against *Leishmania major*, *Leishmania donovani* and other trypanosomatids [15-17]. Despite these encouraging precedents, comparative data on the *in vitro* effect of commercially available CuO NPs (obtained from standardized suppliers such as Sigma-Aldrich) on *Leishmania tropica* are still limited, particularly with side-by-side evaluation on both promastigote and amastigote phases under the same experimental conditions. The present study was therefore designed to fill part of this gap. It aims to evaluate the cytotoxic effectiveness of CuO NPs on *L. tropica* parasites in both promastigote and amastigote stages, and to compare their activity with the standard drug pentostam under *in vitro* conditions, using the MTT colorimetric assay and inhibitory concentration (IC₅₀) analysis as quantitative tools.

Materials and Methods

2.1. *Leishmania tropica* isolate

The *Leishmania tropica* parasite used throughout this study was kindly provided from the laboratory of Al-Zahra Teaching Hospital. The strain was maintained by routine sub-culturing every 1–2 weeks on Novy–MacNeal–Nicolle (NNN) biphasic medium until the experiments were performed.

2.2. Cultivation of *L. tropica* *in vitro*

In order to obtain a sufficient mass of promastigotes, one millilitre of actively growing parasites was transferred from NNN slants to screw-cap vials containing 5 ml of M199 medium (pH 7.0) supplemented with 10 % heat-inactivated foetal calf serum (FCS), and the cultures were kept at 26 °C in a non-CO₂ incubator [18].

For the production of axenic amastigotes, a temperature- and pH-shift protocol with slight modifications was applied. Promastigotes were first inoculated into NNN medium at 26 °C for three days; the cultures were subsequently transferred into M199 medium adjusted to pH 5.0 and supplemented with 10 % FCS, and then incubated at 33 °C for at least five days to allow morphological transformation into the amastigote-like form [18,19].

2.3. Pentostam concentrations

Pentostam (sodium stibogluconate) was used as the positive control drug in this work. Injectable ampoules of pentostam (100 mg/ml) manufactured by Glaxo Operations UK Limited (Member of the GlaxoSmithKline group of companies) were employed. From the original stock solution, seven working concentrations (0.3, 0.6, 0.9, 1.2, 1.5, 1.8 and 2.1 µg/ml) were prepared in sterile distilled water immediately before each experiment, and used in the antipromastigote and antiamastigote assays.

2.4. Copper oxide nanoparticles (CuO NPs) preparation

Copper oxide nanoparticles (CuO NPs, < 50 nm particle size, ≥ 99 % purity, CAS No. 1317-38-0, product number 544868) were purchased from Sigma-Aldrich Chemical Company (Merck KGaA, Darmstadt, Germany). A stock suspension was freshly prepared by dispersing a weighed amount of CuO NPs in sterile distilled water and sonicating the mixture at 100 W and 40 kHz for 40 minutes, in order to break up agglomerates and obtain a homogeneous suspension. The suspension was kept at room temperature, protected from light, and re-sonicated for a further 10 minutes immediately before each experiment. From this stock, the same series of working concentrations used for pentostam (0.3, 0.6, 0.9, 1.2, 1.5, 1.8 and 2.1 µg/ml) was prepared just before being added to the parasite cultures, both in the antipromastigote and the antiamastigote assays.

2.5. Viability of *Leishmania tropica* *in vitro* (MTT assay)

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; thiazolyl blue] is a water-soluble tetrazolium salt that produces a pale yellow solution in its native form. When taken up by viable cells, dissolved MTT is reduced to an insoluble purple formazan product through the cleavage of the tetrazolium ring by mitochondrial and cytosolic dehydrogenase enzymes [20]. The water-insoluble formazan is then

solubilised using dimethyl sulfoxide (DMSO), and the resulting coloured solution is measured spectrophotometrically; the absorbance recorded is directly proportional to the number of metabolically active cells in the sample [21].

The relative number of living parasites was estimated from the optical absorbance values of treated wells, untreated wells, and blank wells, using the following relationship:

$$\text{Viable cells (\%)} = [(AT - AB) / (AC - AB)] \times 100$$

where **AT**, **AC** and **AB** denote the absorbance of the treated samples, the untreated samples (parasite control), and the blank wells, respectively [22].

2.6. MTT assay protocol

Both stages (promastigote and amastigote) of *Leishmania tropica* were adjusted to a final density of 1×10^4 parasites/ml. Equal volumes of parasite suspension and the test compounds (CuO NPs or pentostam at the seven concentrations) were dispensed into flat-bottom 96-well microtiter plates so as to give a final volume of 100 μ l per well. The plates were then incubated at 25 °C for 72 hours. After incubation, 10 μ l of MTT solution was added to each well to give a final concentration of 0.5 mg/ml, and the plates were re-incubated for an additional 4 hours at 25 °C in the dark. The supernatant was carefully removed, and 100 μ l of DMSO was added to dissolve the formazan crystals. The plates were placed on a microplate shaker for gentle agitation, left to stand for 15 minutes, and the absorbance was finally read at 490 nm using an ELISA microplate reader. All experiments were carried out in triplicate.

2.7. Statistical analysis

Data were analysed by the Statistical Analysis System program (SAS, version 9.1, SAS Institute Inc., Cary, North Carolina, USA). The least significant difference (LSD) test was applied to compare between mean values, and differences were considered significant at $p < 0.05$ [23]. The IC_{50} (the concentration at which the parasite proliferation is inhibited by 50 %) was determined for CuO NPs and pentostam on both promastigotes and amastigotes by plotting the logarithm of the drug concentration on the x-axis versus the percentage of viable parasites relative to the control on the y-axis, then applying linear interpolation between the two adjacent points that flank the 50 % viability line [24,25]. The calculations were performed using Microsoft Excel.

Results and Discussion

The present investigation was carried out to assess the effectiveness of CuO NPs, purchased from Sigma-Aldrich, on the viability of *Leishmania tropica* promastigotes and amastigotes *in vitro*, in comparison to the standard antileishmanial drug pentostam. The MTT colorimetric assay was used as the principal tool for measuring parasite viability, and the IC_{50} parameter was used to express the cytotoxic potency of each compound.

3.1. Cytotoxic effect of CuO NPs on *L. tropica* promastigotes

The results obtained after 72 hours of exposure showed clear and statistically significant differences ($p < 0.05$) between all tested concentrations. The number of viable promastigotes decreased in a regular and dose-dependent manner as the concentration of CuO NPs rose. The greatest cytotoxic effect (26.14 ± 0.71 % viability) was seen at the highest concentration (2.1 μ g/ml) while pentostam at the same concentration showed viability of (68.79 ± 0.42 %). In contrast, CuO NPs killed almost three-quarters of the promastigotes at the highest dose tested and pentostam left over two-thirds of parasites alive. The whole pattern of response is summarised in Table 1.

Table 1. The viability percentage of *L. tropica* promastigotes exposed to CuO NPs and pentostam by MTT assay after 72 hr.

Drug concentration (µg/ml)	CuO NPs (% viable cells)	Pentostam (% viable cells)	LSD value
0.3	78.45 ± 1.62	90.78 ± 1.21	8.214 *
0.6	74.23 ± 1.85	89.14 ± 1.07	8.467 *
0.9	68.91 ± 2.14	87.62 ± 1.43	8.039 *
1.2	60.18 ± 1.36	84.07 ± 1.78	8.624 *
1.5	52.80 ± 2.08	81.46 ± 1.62	8.157 *
1.8	38.00 ± 1.55	75.83 ± 0.94	8.392 *
2.1	26.14 ± 0.71	68.79 ± 0.42	8.741 *
LSD value	8.853 *	7.198 *	----
* Significant at ($p < 0.05$).			

The antiparasitic activity of copper-based nanostructures was already indicated by some previous studies. Hashemi et al. [15] reported that green-synthesised copper nanoparticles were effective in inhibiting the growth of *Leishmania major* amastigotes with a concentration dependent manner, especially when associated with meglumine antimoniate. Similarly, Mahmoudv and et al. [26] showed that CuO NPs have considerable activity against *Leucocytozoon*, attributed primarily to the release of copper ions and damage to the parasite membranes. This trend is in line with the picture of viability and proliferation of promastigotes observed in the present work, which showed a dose-dependent leishmanicidal effect of CuO NPs.

On the other hand, the IC_{50} value was computed for both CuO NPs and pentostam against promastigotes. For CuO NPs the IC_{50} value, obtained by linear interpolation between the data points at 1.5 and 1.8 µg/ml, was 1.553 µg/ml after 72 hr (Figure 1). In contrast, pentostam viability remained above 50 % even at the highest concentration tested (68.79 % at 2.1 µg/ml), so the dose-response curve never crossed the 50 % line and no IC_{50} value could be calculated for the antimonial drug under the present experimental window (Figure 2).

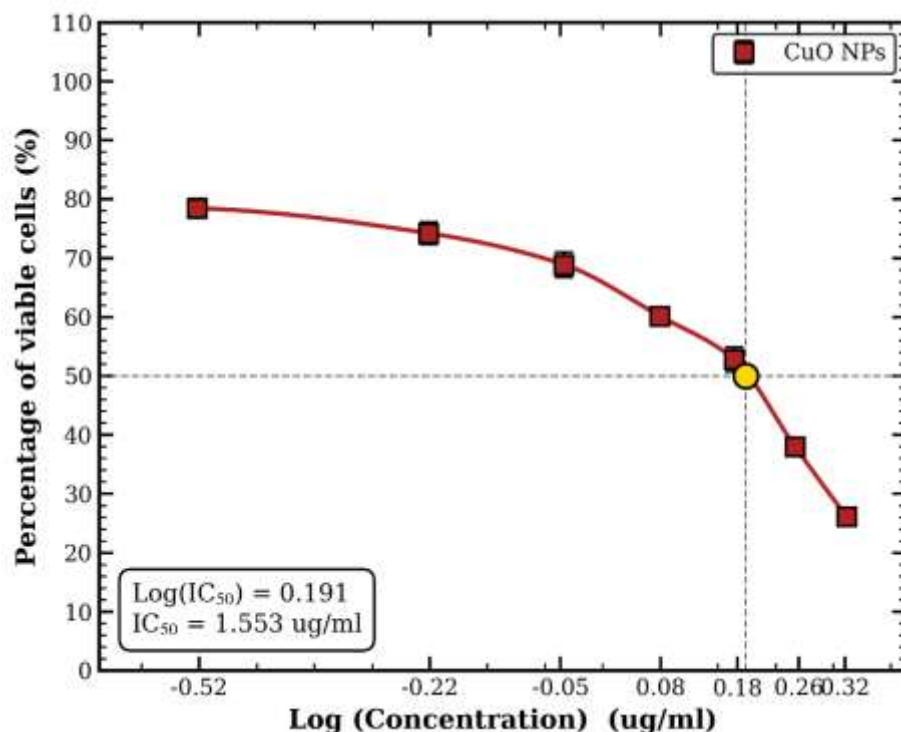


Figure 1. The IC_{50} of CuO NPs on *L. tropica* promastigotes by the MTT assay after 72 hr. The yellow circle marks the intersection of the dose-response curve with the 50 % viability line. $\text{Log}(IC_{50}) = 0.191$, $IC_{50} = 1.553 \mu\text{g/ml}$. Each point represents the mean $\pm$ SD of three independent replicates.

Two general features stand out from Figure 1. First, the curve descends in a smooth, monotonic way as the concentration of the nanoparticles increases, which is a clear indication of a dose-dependent cytotoxic effect: every increment in drug concentration produces a further reduction in the number of live parasites. Second, the slope of the curve is not uniform across the whole range. At low concentrations (0.3–0.9 $\mu\text{g/ml}$) the loss of viability is moderate, whereas in the middle of the range (1.2–1.8 $\mu\text{g/ml}$) the curve drops very sharply, signalling that a critical threshold has been crossed at which the antioxidant capacity of the parasite is overwhelmed. The IC_{50} value of 1.553 $\mu\text{g/ml}$ places the present batch of Sigma-Aldrich CuO NPs in the same order of magnitude as silver and zinc-oxide nanoparticles reported in recent literature for *Leishmania tropica*, making them a competitive candidate for further antileishmanial screening.

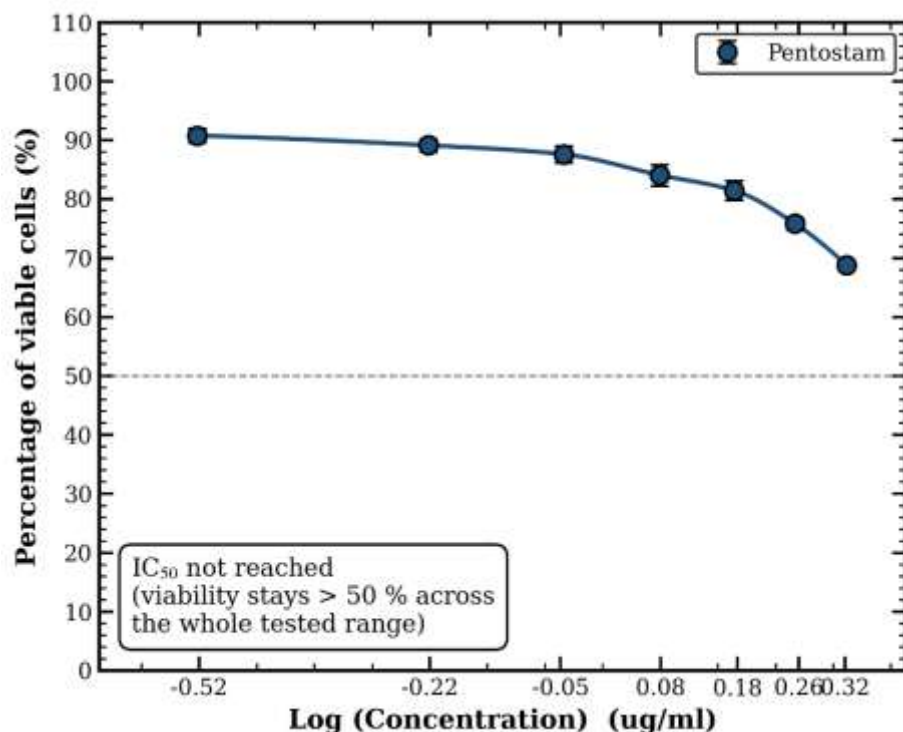


Figure 2. The IC_{50} of pentostam on *L. tropica* promastigotes by the MTT assay after 72 hr. The viability curve does not cross the 50 % line within the tested concentration range, so no IC_{50} value can be calculated. Each point represents the mean $\pm$ SD of three independent replicates.

The contrast between Figure 1 and Figure 2 is striking. The pentostam curve is much flatter and lies, at every concentration tested, well above the 50 % viability line. Even at the highest dose used in this experiment (2.1 $\mu\text{g/ml}$), the viability of the promastigote population was still 68.79 ± 0.42 %, meaning that more than two thirds of the parasites remained metabolically active and viable after 72 hr of drug exposure. This poor in vitro performance of pentostam is not, in itself, unexpected. It is now well established that pentavalent antimonials act mainly as prodrugs that require enzymatic reduction to their trivalent form (Sb (III)) inside the host macrophage in order to display full antiparasitic activity. In axenic cultures of promastigotes, where macrophages are absent, this metabolic activation does not occur, and the apparent potency of pentostam is therefore much lower than that observed in clinical practice [27].

The clearly higher antileishmanial activity of CuO NPs over pentostam observed here is likely related to several interconnected phenomena. First, the very small particle size (< 50 nm in the present batch) gives the nanoparticles a high surface area and the ability to interact directly with the plasma membrane and flagellar pocket of the parasite [28]. Second, partial dissolution of CuO inside the slightly acidic environment of the parasite vacuoles releases Cu^{2+} ions, which catalyse Fenton-like reactions and generate hydroxyl radicals at the cell interior [29]. Third, the redox cycling between Cu(I) and Cu(II) drives a continuous flow of ROS, including superoxide and hydrogen peroxide, which exceed the antioxidant capacity of Leishmania and lead to lipid peroxidation, DNA fragmentation, and mitochondrial collapse [11,12]. These mechanisms operate simultaneously, which probably explains why even relatively low concentrations of CuO NPs were enough to compromise the viability of the promastigote stage.

Other authors have shown that the antimicrobial activity of metal-oxide nanoparticles is closely linked not only to their concentration but also to their morphology, size distribution, surface charge, and surface chemistry [30]. The relatively narrow size distribution and reproducible behaviour of the Sigma-Aldrich CuO NPs employed in the present work is desirable for therapeutic screening, and may be responsible for the smooth monotonic dose-response curve (Figure 1).

3.2. Cytotoxic effect of CuO NPs on *L. tropica* amastigotes

The amastigote form, which is the clinically relevant stage in mammalian hosts, was tested under the same conditions. The results, shown in Table 2, indicate that CuO NPs produced a much stronger cytotoxic effect on amastigotes than pentostam over the whole concentration range. Statistically significant differences ($p < 0.05$) were obtained between the two compounds at every concentration tested. The lowest viability was recorded at 2.1 $\mu\text{g/ml}$ of CuO NPs (5.62 ± 0.51 %), meaning that more than 94 % of the amastigote population had been killed at this concentration. In sharp contrast, pentostam at the same concentration still left 51.06 ± 0.79 % of the amastigotes alive.

Table 2. The viability percentage of *L. tropica* amastigotes exposed to CuO NPs and pentostam by MTT assay after 72 hr.

Drug concentration ($\mu\text{g/ml}$)	CuO NPs (% viable cells)	Pentostam (% viable cells)	LSD value
0.3	76.34 ± 1.85	84.16 ± 1.38	4.508 *
0.6	68.27 ± 1.62	81.59 ± 1.42	6.124 *
0.9	58.27 ± 1.78	79.43 ± 1.55	8.213 *
1.2	44.74 ± 1.34	74.21 ± 1.27	7.405 *
1.5	28.18 ± 1.42	68.74 ± 1.61	8.092 *
1.8	13.95 ± 1.18	58.18 ± 1.72	8.346 *
2.1	5.62 ± 0.51	51.06 ± 0.79	8.927 *
LSD value	6.812 *	7.354 *	----
* Significant at ($p < 0.05$).			

On the other hand, the IC_{50} value was also calculated for CuO NPs and pentostam on the amastigote stage. CuO NPs gave a clear IC_{50} of 1.073 $\mu\text{g/ml}$ after 72 hr (Figure 3), which is appreciably lower than the IC_{50} obtained against promastigotes (1.553 $\mu\text{g/ml}$). This indicates that the amastigote form was actually more sensitive to CuO NPs than the promastigote form, which is a desirable property for any candidate antileishmanial drug, since amastigotes are the parasitic form that drives the pathology of cutaneous and visceral disease. Pentostam, in contrast, did not produce an IC_{50} value within the tested range; its dose-response curve remained above 50 % viability throughout the experimental window (Figure 4).

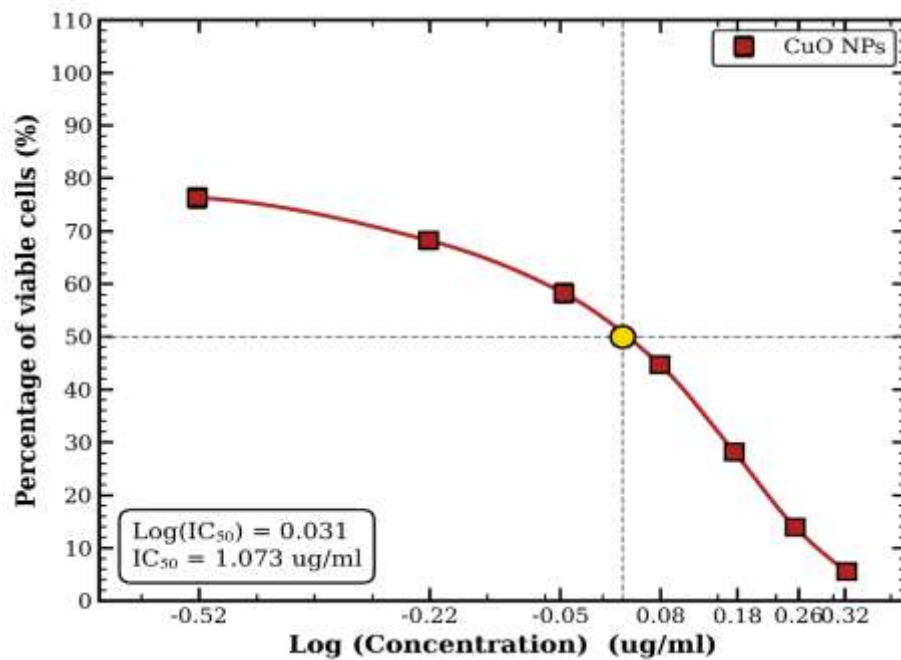


Figure 3. The IC_{50} of CuO NPs on *L. tropica* amastigotes by the MTT assay after 72 hr. The yellow circle marks the intersection of the dose-response curve with the 50 % viability line. $\text{Log}(IC_{50}) = 0.031$, $IC_{50} = 1.073 \mu\text{g/ml}$. Each point represents the mean $\pm$ SD of three independent replicates.

A possible explanation for the higher sensitivity of amastigotes lies in their physiological context. Amastigotes normally reside inside the acidic environment of the macrophage phagolysosome ($\text{pH} \approx 4.5\text{--}5.5$). At this pH, the dissolution of CuO and the release of Cu^{2+} ions are favoured, which intensifies the local oxidative stress experienced by the parasite [31]. In addition, the amastigote form possesses a thinner, less rigid surface coat than the promastigote, and its smaller volume means that any ROS-mediated damage rapidly affects a higher proportion of the cellular machinery. Similar observations have been reported by Jebali and Kazemi [32], who found that several metal-oxide nanoparticles (Ag, Au, TiO_2 , ZnO and MgO) act more efficiently against intracellular *Leishmania* amastigotes than against the extracellular promastigote stage.

It is also useful to remember that *Leishmania* is intrinsically sensitive to ROS. The parasite normally circumvents the macrophage oxidative burst by inhibiting the assembly of the NADPH oxidase complex on the phagosomal membrane [33]. Nanoparticle-mediated ROS, however, are produced non-enzymatically at the particle surface, so they cannot be blocked by this parasite-specific evasion mechanism. The combination of a thin amastigote envelope, an acidic environment that accelerates Cu^{2+} release, and a non-enzymatic source of ROS provides a credible mechanistic basis for the observed potency of CuO NPs on the amastigote stage, as illustrated by the steep mid-range slope of the curve in Figure 3 [13,14,34].

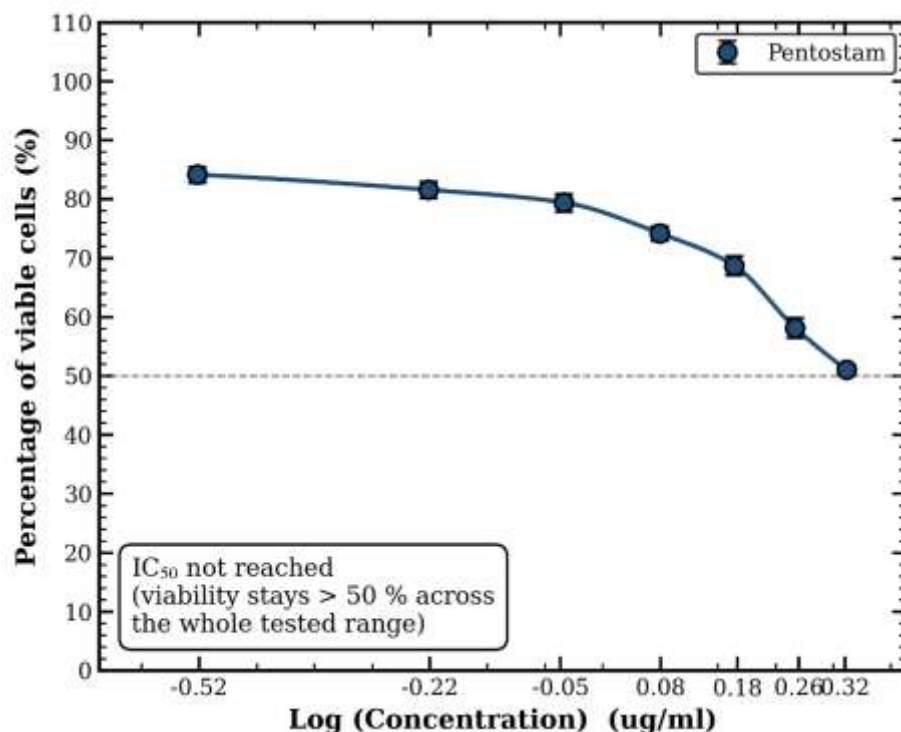


Figure 4. The IC_{50} of pentostam on *L. tropica* amastigotes by the MTT assay after 72 hr. The viability curve does not cross the 50 % line within the tested concentration range, so no IC_{50} value can be calculated. Each point represents the mean $\pm$ SD of three independent replicates.

Figure 4 confirms the same pattern observed for promastigotes: pentostam, when used alone in an axenic culture system and without the metabolic activation provided by host macrophages, fails to bring the viability of the parasite population below the 50 % cut-off. The shape of the curve, although gently descending, does not allow an IC_{50} value to be obtained from the present concentration range. This finding is in line with the growing body of evidence that questions the *in vitro* potency of pentavalent antimonials and emphasises the urgent need for alternative agents [27].

From a comparative perspective, our IC_{50} values (1.553 $\mu\text{g/ml}$ against promastigotes and 1.073 $\mu\text{g/ml}$ against amastigotes) sit in a range comparable to, or even better than, several recently published nanomaterials, including iron-oxide and zinc-oxide nanoparticles tested against *L. tropica* [35,36]. This relative potency is encouraging, particularly since the CuO NPs used here are commercially available, well-characterised, and do not require any specific synthesis protocol that could be a barrier in routine laboratory settings. Finally, it should be noted that copper is an essential trace element for human physiology, and the limited concentrations of CuO NPs needed to kill *Leishmania* parasites in our work (1–2 $\mu\text{g/ml}$) are far below the concentrations that show meaningful cytotoxicity on mammalian cells in published studies [11]. This widens the therapeutic window of CuO NPs and supports their further evaluation as candidates in antileishmanial drug development, ideally with *in vivo* confirmation on animal models of cutaneous leishmaniasis.

4. Conclusions

On the basis of the present results, several conclusions can be drawn. First, the standard antimonial drug pentostam showed only modest activity against *L. tropica* in the *in vitro* tested range, and never reached 50 % inhibition on either promastigotes or amastigotes within the experimental window. Second, copper oxide nanoparticles (CuO NPs) supplied by Sigma-Aldrich exerted a strong and clearly dose-dependent leishmanicidal effect on both stages of the parasite, with IC_{50} values of 1.553 $\mu\text{g/ml}$ against promastigotes and 1.073 $\mu\text{g/ml}$ against amastigotes after 72 hr. Third, the amastigote form, which is the disease-causing

stage in the mammalian host, was more sensitive to CuO NPs than the promastigote stage, which is a favourable feature for therapeutic development. The best concentration of CuO NPs in this study, in terms of cell-killing efficiency, was 2.1 µg/ml after 72 hr of incubation. Overall, the data support the view that commercially available CuO NPs may become a useful starting point for the design of alternative antileishmanial agents, and further in vivo studies, along with cytotoxicity evaluation on mammalian cells, are warranted.

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