RESEARCH ARTICLE

# Analysis of the Relative Effectiveness of Curcumin Loaded Chitosan Nanoparticles and Pure Curcumin Compound in the Treatment of Leishmaniasis

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## ABSTRACT

**Aim:** A proficient drug conveyance plan is expected to treat cutaneous leishmaniasis (CL). Regardless of their deficient solvency, significant expense, low assimilation, and rising medication-safe Leishmania spp., pentavalent antimonials and curcumin (CC) may fix leishmaniasis. Drug conveyance frameworks (DDS) might be utilized to treat CL without these worries.

**Methodology:** We tried free CC and Curcumin-stacked nanoparticles produced using chitosan (CC-CNPs) for antileishmanial movement *in-vitro*. Nanoparticles of Chitosan (CNPs) were produced by tripolyphosphate (TPP), which are negatively charged ionic gelation. CC was added to the CNPs mix. The size, surface shape, epitome adequacy (EE), drug stacking content (DLC), and surface charge of the NPs were characterized using various approaches.

The MTT test was performed to evaluate their effectiveness against *Leishmania tropica* promastigotes and axenic amastigotes after characterization.

**Results:** Round CC-CNPs with a typical molecule size of 276 nm, a zeta capability of (+18.74 mV), and 88% embodiment viability were integrated. Following 72 hours, free CC restrained promastigotes and axenic amastigotes parasite load by 64 and 70%, separately, though CC-CNPs repressed parasites by 91 and 86%. CC-CNPs diminished parasite suitability better than free CC. The half-maximal inhibitory focus (IC50) of CC-CNPs was a lot of lower than that of free CC.

**Conclusion:** In a portion-time subordinate way, CC-CNPs showed more antileishmanial action than unbound CC. I*n-vivo* testing is expected for CL nearby treatment with this detailing.

**Keywords:** Curcumin, Leishmanial treatment, Nanoparticles, Chitosan, Time dependant dose.

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## INTRODUCTION

Female phlebotomine sandflies spread leishmaniasis, a typical dismissed tropical parasitic illness.[1] In 2017, the WHO said that this sickness compromises north of a billion groups in 97 endemic nations because of the absence of immunization and treatment.[2] WHO's disregarded tropical diseases' third most lethal parasitic infection is leishmaniasis.[3] The most widely recognized assortment is cutaneous, trailed by mucocutaneous and visceral.[4]

CL is the most widely recognized kind of leishmaniasis, brought about by 15 species that assault uncovered skin.[5] Around the world, 0.7 to 1.2 million CL cases are recorded yearly, with more than 90% in eleven nations: India, Syria, Afghanistan, Iraq, Saudi Arabia, and Brazil.[6] CL can cause gentle self-recuperating sores, scarring skin, or huge sores that deform the skin, like diffused mucocutaneous leishmaniasis (DMCL) and MCL.[7] Anti-leishmanial immunizations are inaccessible; chemotherapy is the sole treatment.[8] The current suggested enemy of leishmanial drugs, including antimonials compounds, miltefosine, amphotericin B, pentamidine, and paromomycin, are costly, harmful, inadequately bioavailable, and have as of late, evolved drug-safe *Leishmania* spp. To battle leishmaniasis, protected, viable, and reasonable new medicines are needed.[9] The utilization of nanoscale materials in medication has recently grabbed analysts' eye for treating irresistible disorders.[10] Nano-drug conveyance frameworks

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(NDDS) target stacked medications to diminish harmfulness and increment adequacy and remedial efficacy.[11] The restorative prescription fixations expected to kill macrophage intracellular parasites might be decreased utilizing drug conveyance strategies. Leishmaniasis drug-nanoparticle structures have been tried *in-vitro* and *in-vivo*.[12,13] The modest expense, simple assembling, and room-temperature solidness of ionic cross-connected polymeric nanoparticles make them a central issue as NDDS.[14] Chitosan nanoparticles' non-poisonousness, biodegradability, and biocompatibility make them well-known in biomedical research.[15] Chitosan polymer is FDA-endorsed for wound dressing.[16] Ionic gelation CNPs have been tried for antibacterial, antiviral, and hostile leishmanial exercises. The emphatically charged NH3+ gatherings of chitosan and its nanoparticles associate with microorganisms' adversely charged cell layers to actuate antimicrobial activity.[17,18]

Concentrates on show that curcumin defers the S-period of the phone cycle, repressing cell development and harming the parasite's membrane.[19] CC causes moderate incidental effects such as loose bowels, migraine, skin rash, unfortunate dissolvability, and decreased absorption.[20]

A liposomal detailing was made to treat prescription narrow-mindedness or poisonousness from customary CC conveyance and medication obstruction. It has been displayed to work in CL patients and different diseases in liposomal CC. Nonetheless, significant expense, cold chain prerequisite, and drug content change during capacity restricted its clinical use.[21]

This paper portrays the creation, portrayal, and *in-vitro* antileishmanial action of chitosan-covered CC. Our *in-vitro* tries showed that CC-CNPs decrease parasite essentialness more than free CC against *L. tropica*promastigotes and axenic amastigotes.

## MATERIAL AND METHODS

### Methods

Sigma-Aldrich US provided compound RPMI 1640, M199, and culture medium. Acidic corrosive, TPP, and chitosan polymer (Sub-atomic weight) (MW, 120 kDa) were procured from SulabfineChem, Gujarat, India. Curcumin was acquired from VyankateshNatral Concentrates, Chindwada, India. DMSO and MTT reagent were bought from LobaChem, Mumbai, India. Trehalose 100PH came from Mumbai-based Barentz Gathering. Any remaining solvents and mixtures were logical grade and neighborhood.

### Parasites Cultivation

The field-tried culture of *L. tropica* was gained from the Biotech Division at NRI College, which is situated in Bhopal, India. Promastigotes of *L. tropicalis* were refined in RPMI 1640 mechanism for seven days at a temperature of 24 degrees Celsius.[22] This medium included 10% intensity inactivated FBS, 1% penicillin (100 U/mL), and 1% streptomycin (100 mg/mL) arrangement.

### Formulation of CC-CNPs

Ionic gelation was the strategy that was utilized to produce Chitosan nanoparticles,[23] as was referenced before. To fabricate CNPs, changing measures of chitosan polymer at groupings of 1, 2 and 3 mg/mL were broken down in an acidic corrosive arrangement containing 1% volume by volume. The TPP arrangement (0.75 mg/mL) was added to the chitosan arrangement, which was then blended on an attractive stirrer and put away for the time being at 25°C. The following day, 15 minutes of sonication were performed after the short-term hatching. CC-CNPs were made by whirling attractively at room temperature while adding TPP arrangement each drop in turn to a chitosan arrangement that generally contained 4 mg/mL of CC drug. This interaction was completed unexpectedly. A mechanical beating of the arrangement occurred for a sum of four hours. Subsequent to getting the NPs suspensions, they were centrifuged for one hour at a speed of 15000 RPM. Subsequent to being washed with ultrapure water, the nanoparticles were next exposed to the drying system.

### Physical and Chemical Evaluation

*Encapsulation performance and drug delivery*

The CC-CNPs were subjected to centrifugation at a speed of 20,000 revolutions per minute for a duration of 30 minutes at a temperature of 4 degrees Celsius. This was done to evaluate the viability of the encapsulated material (EE) and the amount of drug present (DLC). The experiment was repeated numerous times, and therefore, the mean and standard deviation were not fixed. Subsequently, the EE and DLC of the nano plan were organized with the aid of the following criteria:

**%EE= [(Ac-Bc)/Ac] × 100**

Ac signifies the aggregate sum of CC that was utilized in the development of nanoparticles and is meant in mg, though Bc indicates how much free CC was available in the supernatant and is indicated in mg.

**%DLC= [(Ac-Bc)/Cc] × 100**

Ac is for the all-out volume of CC that was utilized during the combination of nanoparticles and is signified in mg, Bc represents the volume of free CC that was available in the supernatant and Cc represents the complete number of nanoparticles that were available in the supernatant.

### Promastigotes Potential Cytotoxicity Investigation

The tetrazolium color test, frequently known as the MTT, was used to concentrate on the cytotoxic impacts of CC-CNPs on *L. tropica*promastigotes. Fixed-stage promastigotes were cultivated at a convergence of 107 cells/mL in 96-well plates and afterward treated with six unique centralizations of CC-CNPs (50, 40, 30, 20, 10, and 5 g/mL) for 24, 48, and 72 hours separately. From that point onward, 5 mg/l of MTT color was moved every single well, and the situation kept on being brooded at 37°C for four extra hours. In the wake of dissolving the purple formazan gems that were delivered in 100 mL of dimethyl sulfoxide (DMSO), they were centrifuged at a speed of 3000 cycles each moment for 5 minutes. An ELISA peruser, otherwise called a Thermo Logical Microplate Peruser, was used to decide the optical thickness (OD) at 570 nm.

Estimations were made to decide the extent of feasible parasites as well as the EC50 levels of both free CC and CC-CNPs. As a proportion of value control, miltefosine was used. Following is the equation that was used to work out the level of practicality.

**Viable cells%: [(T-B) / (C-B)] ×100.**

The absorbance of the treated examples is signified by the letter T, how much assimilation of the pattern test is meant by the letter B, and the frequency of the negative control is indicated by the letter C.

### Evaluations of the Hazardous Potential of Axenic Amastigotes

In this study, we investigated the effects of amphotericin B-CNPs and free amphotericin B against axenic amastigotes of *L. tropica* in a laboratory setting. From the outset, the promastigotes were kept in a hatchery with 5% CO2 at a temperature of 37℃. This outcome in the improvement of axenic amastigotes from promastigotes. Subsequent to being moved to an ELISA plate, the amastigotes were then exposed to a similar degree of CC-CNPs and free CC as the promastigotes.[24-26]

### Statistical Examination

Involving sigmoid portion reaction bends and Diagram Cushion Crystal for Windows rendition 7.0 (both by Chart Cushion Programming, USA), the IC50 values were determined.

## RESULTS AND DISCUSSION

### Synthesis of Nanoparticles

CC-CNP union, chitosan polymer blend, TPP arrangement combination, and CC substance amalgamation were undeniably joined by means of an ionotropic gelation approach. Basically, the CC arrangement was blended in with chitosan, and afterward 1% acidic corrosive combination and TPP were gradually added to the CC-CNPs that were being orchestrated. This response will bring about the development of nanoparticles in an answer that has a light yellow tone (Figures 1a, 1b). Trehalose was thusly brought into the arrangement following centrifugation of the combination.

### Evaluation of CC-CNPs

*Cytotoxicity against promastigotes, as determined by the MTT assay*

A colorimetric MTT test was used to decide if promastigotes are suitable. More or less, *L. tropica* promastigotes went through brooding at 24, 48, and 72 hours, separately, with six proportions (50, 40, 30, 20, 10, and 5 g/mL) of both CC-CNPs and unbound CC. This was finished in three separate tests. Both time and portion are expected for the hindrance of parasite endurance, albeit this peculiarity happens at various moments for stacked and free medications. Notwithstanding this, hatching promastigotes for 72 hours with 50 g/ml of CC-CNPs brings about a 91% decrease in the reasonability of the organic entity (Table 1). What's more, the imperativeness of promastigotes in the wake of being presented to fluctuating weakening of free CC is introduced in Table 2. There was

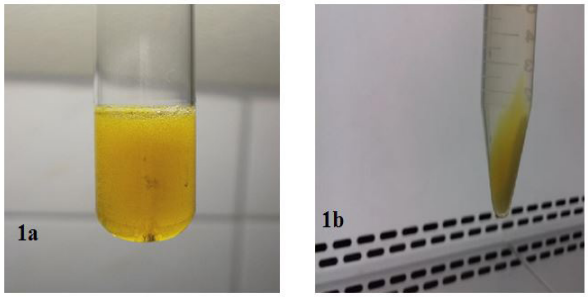


**Figure 1:** 1a) Nanoparticles in solution, 1b) Nanoparticles after centrifugation

**Table 1:** The proportion of viable promastigotes after exposure to CC treatment-CNPs

| *CC-Chitosan NP's Concentration (µg/mL)* | *Promastigotes chances of survival (%)* | | |
|---|---|---|---|
| | *24 hours* | *48 hours* | *72 hours* |
| 50 | 49 | 33 | 09 |
| 40 | 54 | 49 | 19 |
| 30 | 65 | 53 | 24 |
| 20 | 68 | 56 | 27 |
| 10 | 70 | 58 | 31 |
| 05 | 79 | 63 | 37 |

a 36% opportunity of endurance for promastigotes patients after treatment with an ordinary CC drug. Following 24, 48, and 72 hours of brooding, the CC-CNPs had IC50 upsides of 0.1275 and 0.3810 g/mL against promastigotes and amastigotes, individually. In correlation, the IC50 upsides of free CC against *L. tropica* promastigotes and axenic amastigotes were 0.5427 and 0.6024 g/mL, separately.

### The effects of leishmanicidal agents on axenic amastigotes

What's more, the ramifications of CC-CNPs and free CC on axenic amastigote development *in-vitro* against *L. tropica*is at 37℃ and 5% carbon dioxide were examined. At a temperature of 37℃ and a pH of 5.5, a culture of axenic amastigotes was kept in a climate containing six weakenings (40, 30, 20, 10, 5, and 2.5 g/mL) of CC-CNPs and free CC for 24, 48, and 72 hours. The cytotoxicity of CC-CNPs as estimated in opposition to *L. tropica* axenic amastigotes at a scope of various brooding spans is introduced in Table 3. The most extreme decrease in parasite endurance accomplished by CC-CNPs against

**Table 2:** The survival rate of promastigotes following treatment with a pure curcumin medication

| *CC-Compound Concentration (µg/mL)* | *The viability percentage of promastigotes (%)* | | |
|---|---|---|---|
| | *24 hours* | *48 hours* | *72 hours* |
| 50 | 67 | 49 | 36 |
| 40 | 70 | 57 | 40 |
| 30 | 75 | 60 | 42 |
| 20 | 79 | 63 | 45 |
| 10 | 82 | 68 | 54 |
| 05 | 85 | 70 | 65 |

**Table 3:** The percentages of amastigotes viability that have been treated with CNPs

| *CC-Chitosan NP's Concentration (µg/mL)* | *Amastigotes viability expressed as a percentage* | | |
|---|---|---|---|
| | *24 hours* | *48 hours* | *72 hours* |
| 40 | 53 | 37 | 14 |
| 30 | 60 | 58 | 25 |
| 20 | 70 | 61 | 30 |
| 10 | 74 | 63 | 32 |
| 05 | 77 | 64 | 35 |
| 2.5 | 83 | 67 | 45 |

**Table 4:** The percentages of amastigotes viability that have been treated with pure curcumin compound

| *CC-Compound Concentration (µg/mL)* | *Amastigotes' viability expressed as a percentage* | | |
|---|---|---|---|
| | *24 hours* | *48 hours* | *72 hours* |
| 40 | 69 | 50 | 30 |
| 30 | 73 | 57 | 41 |
| 20 | 75 | 59 | 45 |
| 10 | 82 | 65 | 46 |
| 05 | 83 | 69 | 55 |
| 2.5 | 87 | 71 | 69 |

amastigotes over a brooding time of 72 hours was 84%. This decrease was portion and time-subordinate. Conversely, the life span of hostile to axenic amastigotes is just restrained by 70% when free CC is utilized (Table 4). As per a surmised decrease in parasite endurance, the counter axenic amastigotes movement of CC-CNPs is essentially stronger than that of unadulterated CC.

## DISCUSSION

Nanotherapy may solve leishmanicidal drug difficulties.[29] These include the medications' inability to dissolve in water, the pain of intravenous administration, and their potentially harmful side effects. In this study, we tested nanoparticles made from chitosan loaded with curcumin (CC) and free CC, one of the most effective and potentially life-saving antileishmanial drugs, against *L. tropica*promastigotes and amastigotes *in-vitro*. The compound cycle of deacetylation causes the chitosan polymer to unite. Chitosan polymer is a popular nanotechnology drug carrier. Past research explored its most notable antibacterial, antiviral, antileishmanial, and antifungal activities.[30,31] The method used ionic gelation throughout the CNP union cycle. When cationic chitosan polymer and TPP anions react, cationic nanoparticles (CNPs) form. This method was used to produce most CNPs, according to a recent assessment by Hadidi and partners.[32] This method produces large numbers of nanoparticles, which Lazaridou *et al*. found to be more antimicrobial in 2020. In this study develops a chitosan-based *L. tropica* drug delivery system. The dynamic fixing was chitosan. According to their morphology, CC-CNPs are round and have a mean molecular size of 150 nm. Investigating NPs yielded these data. A previous study[34] found that ionic gelation-delivered medication-stacked CNPs were 200 to 300 nm in size.

Reticuloendothelial cells, which intracellular parasites use as host cells, readily bind and phagocytose nanoparticles above 200 nm.[35] Because intracellular parasites target reticuloendothelial cells, this is crucial. The EE and DLC of CC-CNPs were 88 and 48%, respectively. These finds match those from prior explorations.[36, 37]

In the current review, a correlation was led in regards to the ward upon time and portion related inhibitory activities of CC-CNPs, as well as those of free CC, on the two types of *L. tropica*. This was finished to decide if sort of CC-CNPs were more compelling. When contrasted with the IC50 values with the expectation of complimentary CC, which were 0.5427 and 0.6024 micrograms per milliliter, separately, the IC50 values for CC-CNPs were 0.1275 and 0.3810 micrograms per milliliter. There have been various fruitful examinations completed in the past determined to assess drug-stacked nanoparticles as a treatment for leishmaniasis.[38,39] These examinations have been done. Patients experiencing leishmaniasis were given nanoparticles as a sort of treatment in these examinations. Mostafavi *et al*. as of late completed a concentrate in which they stacked the Amphotericin B medication onto pernicious loaded selenium nanoparticles and inspected its cytotoxicity *in-vitro* against *L. tropica*.[40] In this review, the scientists observed that the medicine was successful in restraining the development of *L. tropica*. When assessed *in-vitro* conditions, they found that the impacts of free amphotericin B and amphotericin B-stacked dangerously varied relying upon the dose as well as how much time that had elapsed.

Mehrizi *et al*. identified the detrimental impacts of amphotericin B stacking dendrimers, betulinic acid, and chitosan on *L. tropicalis*. The process of drug absorption from the gastrointestinal system is very varied, making it difficult to achieve prolonged gastric retention of the dose form.[41] Casa *et al*. investigated the inhibitory effects of serum egg white nanoparticles derived from cows, which were loaded with amphotericin B, and compared them to the effects of free amphotericin B medicine. The current work has successfully developed dutasteride-loaded microsponges that exhibit excellent compatibility with other components. This formulation significantly enhances the solubility of the medication without any interference from other components.[42] They discovered that the drug administered in a layered manner had superior results compared to the therapy given without any restrictions. Ammar *et al*. discovered that the toxicity of nanoparticles combined with amphotericin B medicine was much greater than that of the free CC medicament.[43-45]

## CONCLUSION

As per the discoveries of the ongoing examination, *in-vitro* destruction of *L. tropica* can be worked with by a medication conveyance framework that depends on chitosan. Chitosan nanoparticles were synthesized via the ionic gelation technique, which entailed encapsulating CC inside them. The CC-CNPs

that were made showed a moderately restricted normal size scope of 276 nm, a high epitome viability of 88%, and a generally consistent positive zeta capability of +18.74 mV. Then again, the information on the level of medication discharge shows an effect that impedes the medication's delivery. The newly conducted nano-definition showed significant antileishmanial activity when tested on promastigotes and axenic amastigotes obtained from *L. tropica*. The IC50 value of the CC-CNPs was lower than that of the free CC compound. The IC50 values of CC-CNPs against promastigotes and amastigotes were significantly reduced compared to ordinary CC and the control prescription miltefosine. When compared to free CC medicines, CC-CNPs exhibited much higher antileishmanial activity, as shown by a reduction in the vitality of the parasites. In summary, CC-CNPs have the potential to serve as a potent alternative therapeutic option for eliminating Leishmania parasites that have developed resistance to current pharmacological therapies.

## AUTHORS' CONTRIBUTIONS

Concept – S. M; Design – G.S.M; Supervision – S. M; Resources – S. M; Materials – G. P; Data Collection and/or Processing – P. Y; Analysis and/or Interpretation – G. S. M;Literature Search – G.S.M; Writing – S. R; Critical Reviews – S. M;All authors read and approved the final manuscript."

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