



ORIGINAL ARTICLE

Anti-proliferative and apoptosis induction activities of curcumin on *Leishmania major*



Maha Elamin^{a,*}, Ebtsam Al-Olayan^a, Rewaida Abdel-Gaber^{a,b}, Ramy S. Yehia^c

^a Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

^b Department of Zoology, Faculty of Science, Cairo University, Cairo 12613, Egypt

^c Department of Botany and Microbiology, Faculty of Science, Cairo University, Cairo 12613, Egypt

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KEYWORDS

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Abstract Leishmaniasis is a major vector-borne disease triggered by an obligate intracellular protozoan parasite of the genus *Leishmania* and transmitted by the bite of phlebotomine female sand flies. This parasite causes a wide range of human diseases, from localized self-healing cutaneous lesions to fatal visceral infections. The aim of this study was to investigate the cytotoxic, antiproliferative, and apoptotic effects of curcumin on *Leishmania major* promastigotes (MHOM/SA/84/JISH) and to assess these effects on the cell cycle of promastigotes. The MTT colorimetric assay was used to evaluate the cytotoxicity and proliferation of promastigotes. Additionally, flow cytometry was used to analyze the cell cycle. The Annexin V/propidium iodide staining technique followed by flow cytometry was used to study the cell death induced by curcumin. In this study curcumin showed a potent antileishmanial effect, exhibiting cytotoxicity against *L. major* promastigotes. At 80 μ M, the survival in curcumin treated promastigotes reached 22%; however, the median lethal concentration of curcumin (LC₅₀) was 35 μ M. The drug exerted its cytotoxic effect by inducing apoptosis. Curcumin-induced cell death in promastigotes reached 82.5% at 80 μ M concentration. In addition, curcumin delayed the cell cycle in the S-phase inhibiting cell proliferation. Thus, curcumin was shown to be effective against *L. major* promastigotes. Therefore, curcumin merits further research studies to demonstrate its efficacy in treating cutaneous leishmaniasis.

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* Corresponding author.

E-mail address: malamyn@ksu.edu.sa (M. Elamin).

PALABRAS CLAVE

Curcumina;
Leishmania major;
 Citotoxicidad;
 Apoptosis;
 Proliferación celular

Efectos de inducción antiproliferativa y apoptótica de la curcumina en *Leishmania major*

Resumen La leishmaniasis es una enfermedad vectorial significativa, obligadamente desencadenada por un protozoo intramacrófago del género *Leishmania*, que se transmite por la picadura de mosquitos flebótomos hembra. Esta especie parasitaria causa una gran variedad de enfermedades humanas: desde lesiones cutáneas localizadas autocurables hasta infecciones viscerales mortales. El objetivo de este estudio fue investigar los efectos citotóxicos, antiproliferativos y apoptóticos de la curcumina en los promastigotes de *Leishmania major* (MHOM/SA/84/JISH) y evaluar su efecto en el ciclo celular de este parásito. Se utilizó el ensayo colorimétrico MTT para evaluar la citotoxicidad y la proliferación de los promastigotes. Además, se utilizó la citometría de flujo para analizar su ciclo celular. Se empleó la técnica de tinción de yoduro de propidio/anexina V y, posteriormente, citometría de flujo para estudiar la muerte celular inducida por la curcumina. En este estudio, la curcumina reflejó un potente efecto leishmanicida. También exhibió citotoxicidad frente a los promastigotes de *L. major*. A una concentración de 80 μM , la supervivencia de los promastigotes tratados con curcumina fue del 22%; en tanto que la concentración letal media (LC_{50}) fue de 35 μM . El fármaco produjo su acción parasiticida mediante la inducción de apoptosis. La muerte celular inducida por curcumina en los promastigotes fue del 82,5% a una concentración de 80 μM . Además, la curcumina demoró el ciclo celular en la fase S e inhibió así la proliferación celular. La curcumina demostró efectividad frente a los promastigotes de *L. major* y, por tanto, consideramos que los estudios deberían continuar a fin de demostrar su efectividad para tratar la leishmaniasis cutánea.

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Introduction

Leishmaniasis is a vector-borne disease triggered by a heterogeneous group of protozoa that belongs to the *Leishmania* genus¹⁵. This disease constitutes an emergent threat, with elevated morbidity and mortality levels, and has three main forms: cutaneous, mucocutaneous, and visceral⁵. Leishmaniasis is a common condition that affects 12 million people worldwide with an approximate 1–2 million new cases arising annually². The cutaneous form is expected to affect around 10 million people³⁹. The broad geographical distribution is mainly due to the constant increase in the risk factors of leishmaniasis, including migration, environmental changes, deforestation, urbanization, immunosuppression, and malnutrition. Consequently, leishmaniasis represents a potential threat to several areas²⁷. The lack of effective vaccines, the problems with vector control and the development of drug resistance have also raised the incidence of this disease⁵. The most commonly used medications for leishmaniasis treatment are pentavalent antimonials, amphotericin B, paromomycin, and pentamidine, which have severe side effects, require high doses for long periods of time, and are administered parenterally²⁸. An effective drug would be beneficial to overcome the complications caused by leishmaniasis chemotherapy if it delivers safe care at a reasonable cost. Recently, phytotherapy has received considerable attention as an alternative to chemotherapy in the treatment of parasitic diseases⁹.

Curcumin (diferuloylmethane) is one of the most commonly characterized phytochemicals. It is a yellow natural product extracted from the rhizome of *Curcuma longa* (turmeric) that is used as food spice and colorant. Curcumin is a polyphenolic, non-toxic and pharmacologically active substance that has antioxidant, anti-inflammatory and antiparasitic activities. For centuries, it has been used as a therapeutic agent against different diseases²¹. Antiprotozoal activities of curcumin have also been identified both *in vitro* and *in vivo* for *Plasmodium*¹², *Leishmania*^{13,22,34}, *Trypanosoma*²⁶, and *Giardia lamblia*²⁹. Curcumin was able to reduce parasitemia by 80–90% in mice infected with *Plasmodium berghei*³³. Curcumin has various anticancer properties including the inhibition of cell proliferation and angiogenesis as well as cell death induction in tumor cells¹. Alternative treatments, including dietary supplements and herbal remedies, are increasingly of interest today. The multiple health benefits and medicinal properties of curcumin attributed to its antioxidant and anti-inflammatory effects have received worldwide attention. Curcumin has a long established safety record, it has been accepted as a safe compound without toxic effect by the FDA. The allowable daily intake of curcumin is 0–3 mg/kg body weight¹⁹. Up to 12 g/day of curcumin consumption has been shown to have no harmful effect on individuals¹⁴. In the present study an attempt was made to evaluate curcumin's leishmanicidal, antiproliferative and apoptotic effect as well as its capacity to delay the cell cycle in the S-stage on *Leishmania major* promastigotes.

Materials and methods

Parasite culture

Leishmania major promastigotes (MHOM/SA/84/JISH)¹⁶ of the Saudi strains were cultured in a complete medium consisting of RPMI-1640 (GIBCO, New York, USA) supplemented with 2 mM L-glutamine, 10 mM HEPES, 24 mM NaHCO₃, 100 U/ml penicillin, 100 µg/ml streptomycin and 10% heat-inactivated fetal bovine serum (Sigma Aldrich, St. Louis, MO, USA). Parasites were incubated at 26 °C in a 5% CO₂ atmosphere (Heracell-1501 CO₂ Incubator, Thermofisher). Promastigotes were collected and used to evaluate the antileishmanial activity of curcumin.

Cell viability measurements by the MTT assay

Different concentrations of curcumin (Sigma Aldrich, St. Louis, MO, USA) were prepared in dimethyl sulfoxide (DMSO). Exponential-phase *L. major* promastigotes in cultured media (1.5×10^6 /ml) were seeded into 96-well plates and treated at the desired concentrations of the drug at final concentrations of 20, 40, 60, 80 µM. DMSO was used to treat the control wells. Blank only wells contained media. All tests were performed in triplicate. Plates were incubated at 26 °C for 48 h in 5% CO₂. A modified MTT colorimetric assay with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Sigma Aldrich, St. Louis, MO, USA) was performed to detect promastigote viability. The MTT reagent (250 µg/ml) was added to each well and the plates were incubated at 26 °C for 4 h. DMSO was added to dissolve the formazan crystals. The amount of cleaved tetrazolium salts to formazan, which is directly correlated with the number of metabolic active cells in culture, was quantified at 540 nm of absorbance using the enzyme-linked immunosorbent assay (ELISA) reader (Synergy LX Multi-Mode Reader, Biotek), according to Mosmann²⁵. Cell viability was calculated on the basis of the equation: absorbance of the treated sample/absorbance of the control cells $\times$ 100. All values are means of triplicate wells. Fifty percent (50%) inhibitory concentration (LC₅₀) according to Branquinho and coworkers⁶ (a drug concentration that reduces the cell growth rate by 50%) was determined from the dose-responsive curve. Results are expressed as the means and standard deviations of three independent experiments, each performed in duplicate.

Annexin V/PI and flow cytometry

Promastigotes were cultured as described before and either treated with DMSO, used as a control or tested with curcumin at 20, 40, 60, 80 µM concentrations. Promastigotes were harvested after 48 h and washed twice in cold PBS, then centrifuged using Heraeus™ Fresco™ 21 Microcentrifuge, Thermofisher. Promastigotes were stained with propidium iodide (PI) and Alexa Fluor 488 Annexin V (Life technologies, Germany) in accordance with the manufacturers' instructions. The percentage of cells was determined by the BD FACSCalibur™ flow cytometer and the Cell Quest Pro software from Becton Dickinson (San Jose, CA, USA). Three

independent experiments were performed for each promastigote culture³⁸.

Cell proliferation measurements by the MTT assay

Promastigotes were cultured and incubated as in the viability assay mentioned above. Curcumin was added to 96-well plates at a final concentration of 80 µM. Plates were incubated at 26 °C in 5% CO₂ at different time intervals (0, 24, 48, 72 h). The MTT reagent was added and the absorbance was measured at 540 nm by the enzyme-linked immunosorbent assay (ELISA) reader. The results were expressed as means and standard deviations of three independent experiments, each of which was performed in duplicate²⁴.

Cell cycle analysis by flow cytometry

Promastigotes were treated with DMSO or curcumin (80 µM) and then collected and re-suspended in 1 ml of Phosphate-Buffered Saline (PBS) before being fixed with a drop of 3 ml of 100% methanol. Fixed promastigotes were centrifuged, re-suspended at 50 µl of RNase (1 mg/ml) and incubated at room temperature for 30 min, followed by the addition of 1 ml of 0.1 mg/ml of propidium iodide (PI)¹¹. The DNA content of the cells was analyzed by flow cytometry (Becton Dickinson). The percentage of promastigotes in various cell-cycle phases was determined using the Cell Quest software (Becton Dickinson).

Statistical analysis

Data were presented as mean and standard deviation using SPSS-17 statistical software. Comparisons between groups were rendered with the Student's t-test. LC₅₀ was obtained through an exponential regression data analysis using the Sigma-plot computer software.

Results

Cytotoxic effect of curcumin on *L. major* promastigotes

The MTT assay was used to investigate the cytotoxic effects of curcumin on *Leishmania major* promastigotes. Figure 1 shows the dose-dependent effect of curcumin on *L. major* promastigotes. The survival percentage of promastigotes of the curcumin-treated groups was statistically compared to the non-treated control group. The results showed that promastigotes were highly sensitive to curcumin, particularly when the dose was increased. The median lethal curcumin concentration (LC₅₀) was 35 µM (Fig. 1). In the absence of curcumin, *L. major* promastigotes survival was 100%, whereas at 20 µM curcumin, promastigotes survival was 75% (Fig. 1). Survival decreased to 41.5% at 40 µM curcumin. At 60 µM, there was a sharp drop in promastigote survival, reaching 25.3%; however, at 80 µM, survival in curcumin-treated promastigotes decreased to 22% (Fig. 1). The difference in survival between curcumin-treated and non-treated promastigotes was highly significant ($p < 0.001$) ($t = 50.7$).

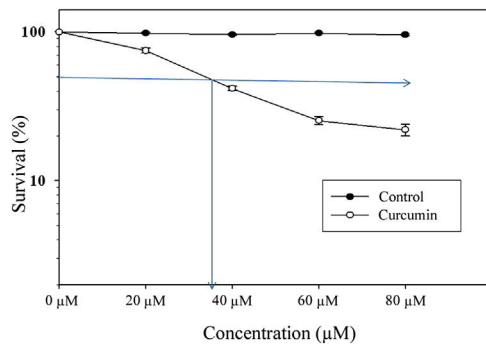


Figure 1 Cytotoxic effect of curcumin on *L. major* promastigotes. Exponentially growing promastigotes were cultured in 96-well plates and treated at the indicated concentrations of curcumin for 48 h. Cell death was analyzed using the MTT assay. The arrows indicate LC50. Error bars represent standard deviations of at least three different experiments.

Apoptotic effect of curcumin on *L. major* promastigotes

In order to study whether the mechanism of cell death triggered by curcumin is due to apoptosis or necrosis, an Annexin V/propidium iodide (PI) staining technique followed by flow cytometry was used. *L. major* promastigotes were treated at different curcumin concentrations for 48 h and then stained and sorted. Fig. 2A shows four groups of cells, viable cells that excluded both Annexin V and PI (Annexin V[−]/PI[−]), bottom left; early apoptotic cells that were only stained with Annexin V (Annexin V⁺/PI[−]), bottom right; late apoptotic cells that were stained with both Annexin V and PI

(Annexin V⁺/PI⁺), top right; and necrotic cells that were only stained with PI (Annexin V[−]/PI⁺), top left. Following the deduction of the proportion of spontaneous apoptosis, the proportion of apoptosis was assumed to be the amount of both early and late apoptosis. Figure 2A and B, exhibits the cytotoxicity of curcumin against *L. major* promastigotes. Importantly, curcumin induced death by apoptosis, this impact increased in a dose-dependent manner. The proportion of apoptotic promastigotes at 20 μM was approximately 24% (Fig. 2A and B). The proportion was 63% in response to 40 μM curcumin; however, curcumin-induced apoptosis was 72% at 60 μM. The maximum proportion of cell death (82.5%) was achieved when promastigotes were treated with 80 μM curcumin (Fig. 2A and B).

Curcumin inhibits *L. major* promastigotes proliferation by delaying the cell cycle at the S phase

The impact of curcumin on *L. major* promastigote proliferation was analyzed using the MTT cell proliferation assay. Figure 3 shows that while the control with non-treated promastigotes continued to proliferate in a time-dependent manner, the number of curcumin-treated promastigotes decreased sharply only after 24 h of treatment and continued to decline after 72 h of treatment. Comparing curcumin-treated with non-treated promastigotes in the control group, there was a highly significant difference in promastigote proliferation after 72 h of treatment ($p < 0.001$) ($t = 15.8$) (Fig. 3).

The effect of this agent on the cell cycle was investigated after demonstrating the inhibitory effect of curcumin on cell proliferation. To this end, *L. major* promastig-

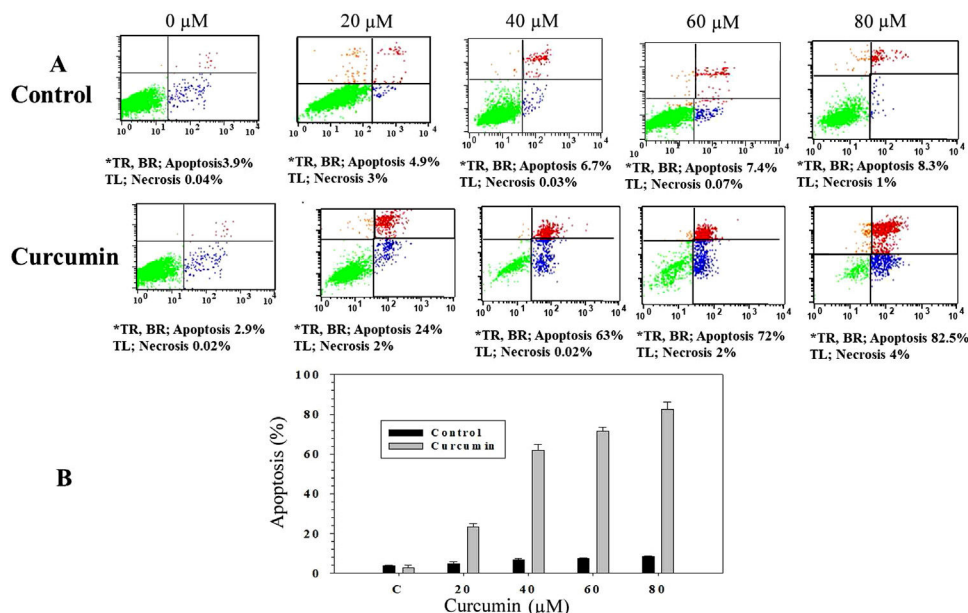


Figure 2 Curcumin triggers apoptosis in *L. major* promastigotes. Promastigotes were either sham-treated or challenged at the indicated concentrations of curcumin for 48 h and then cell death was analyzed using the Annexin V/PI flow cytometry assay. (A) Charts indicating the proportion of apoptotic and necrotic cells. (B) Histogram showing the proportions of apoptotic cells. The difference in apoptosis between curcumin-treated (different doses) and non-treated promastigotes was highly significant ($p < 0.001$). *TR, top right; BR, bottom right; TL, top left.

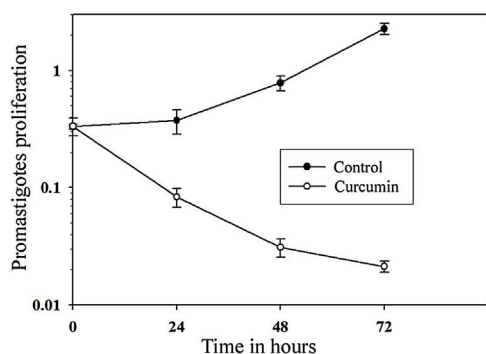


Figure 3 Curcumin inhibits the proliferation of *L. major* promastigotes. Promastigotes were cultured in 96-well plates and were either sham-treated (DMSO) or challenged with curcumin (80 μ M) for the indicated periods of time, and then cell proliferation was assessed by the MTT assay. Error bars represent standard deviations of at least three different experiments.

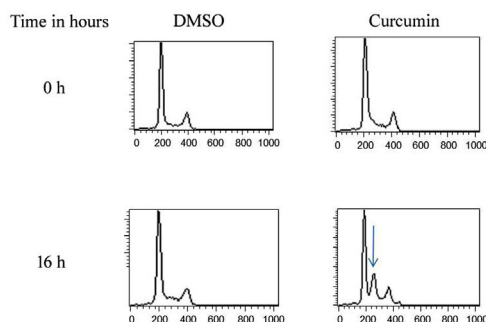


Figure 4 Curcumin inhibits the proliferation of *L. major* promastigotes by delaying cell cycle at S phase. Cells were either sham-treated or challenged with Curcumin (80 μ M) for different time intervals. The cell cycle status was analyzed by flow cytometry. The arrow indicates the accumulated cells at S phase. The curves show the proportions of cells in various phases of the cell cycle as indicated.

otes were either sham-treated or challenged with curcumin (80 μ M) at various time intervals, and then cells were fixed, stained with PI. The cell cycle examined by flow cytometry (Figs. 4 and 5), showed curcumin-dependent cell accumulation in the S-phase reaching, after 16 h of incubation, a maximum level of about 33% compared to 14% in the control group (Figs. 4 and 5).

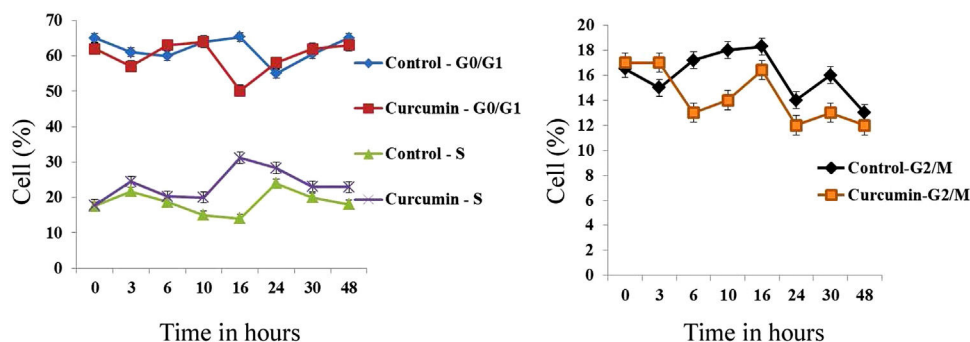


Figure 5 Curcumin inhibits the proliferation of *L. major* promastigotes by delaying cell cycle at S phase.

Discussion

Most drugs currently used for leishmaniasis have several limitations, such as high toxicity, challenging treatment schedules, and development of resistance²⁰. Therefore, there is an urgent need for new, safe, more effective and economically feasible drugs for the treatment of leishmaniasis. Herbal products have been used for the treatment of multiple human diseases for thousands of years. Low specificity, adverse reaction and toxicity of chemotherapeutic agents are commonly observed during chemotherapy treatment. Miltefosine has not been used in this study; it has been used in *Leishmania* treatment in many previous studies. The main safety concerns of miltefosine are related to its effect on the mucosa of the gastrointestinal tract and its potential for teratogenicity. The gastrointestinal side effects of miltefosine include loss of appetite, fatigue, diarrhea, or a combination of these events. Many commonly reported toxic effects associated with miltefosine mainly involve the kidneys and the liver, affecting the activity of the liver, inducing elevated bilirubin, SGOT and SGPT, and causing detrimental effects on kidney function marked by elevated creatinine and serum aspartate aminotransferase³⁰. Curcumin, a natural pharmacologically active agent, has several anti-inflammatory mechanisms that may be detrimental to effective host control of intracellular microbial infections. In our /this report, we have provided clear evidence that curcumin may constitute a potent antileishmanial agent against *L. major* promastigotes *in vitro*.

The present study shows that curcumin has a dose-dependent cytotoxic effect against *L. major* promastigotes. Previously, the leishmanicidal activity of curcumin was dose-dependent^{22,34}. In the present analysis, the median lethal concentration of curcumin (LC₅₀) was 35 μ M, being these results compatible with those from Kodie and coworkers which obtained an LD₅₀ value of 37 μ M for curcumin activity against *L. major* promastigotes *in vitro*²². In a previous report, curcumin displayed LD₅₀ of about 4.5, 5.7, and 5.9 μ M against *L. major*, *Leishmania tropica*, and *Leishmania donovani* promastigotes, respectively, with 100% of curcumin-induced killing at 13.5 μ M³⁴. The maximum parasite killing proportion achieved in this analysis was roughly 80%; however, this percentage, a drug concentration of up to 80 μ M was used to obtain this percentage. The difference in results could be explained by the metabolic variability during the development of *Leishmania* promastigotes in

cultures and variations in different *in vitro* culture conditions. Curcumin has an outstanding safety profile. Indeed, different phase I clinical trials have shown that curcumin is safe when administered at doses up to 12g daily for 3 months^{21,36}. In another study, the dose-dependent death of *L. donovani* promastigotes occurred with curcumin at concentrations between 20 and 60 μ M, reaching 90% when 55 μ M of the drug was used¹³.

The Annexin V/propidium iodide (PI) staining technique was used to confirm the cytotoxic nature of curcumin and to identify the death pathway that this agent triggers in *L. major* promastigotes. The apoptotic activity of curcumin was dose-dependent. Interestingly, the killing function of the drug is by apoptosis and the maximum necrotic death (4%) was reached when promastigotes were administered at the highest dose (80 μ M) of the drug. This could be explained by the fact that curcumin may have the ability to modulate numerous targets and cell signaling pathways, as well as affect gene-regulating apoptosis. In a previous report, we showed that apoptosis was triggered by curcumin via the mitochondrial pathway by downregulation of Bcl-2, a downstream anti-apoptotic effector of the Shh signaling in medulloblastoma cells¹⁷. The pharmacological efficacy of curcumin against *Leishmania* parasites is also unclear. One of the features of metazoan apoptosis is the lack of mitochondrial membrane capacity, which has been reported to play a key role in drug-induced death in protozoans such as *Leishmania*³⁵. Curcumin-induced development of reactive oxygen species (ROS) and elevation of cytosolic calcium have been previously demonstrated, followed by exposure of phosphatidylserine to the outer plasma membrane leaflet and DNA fragmentation in *L. donovani* promastigotes¹³. Using *L. donovani* species, both *in vitro* and *in vivo*, leishmanicidal activity was induced by nanoformulation of curcumin alone; however, curcumin showed *in vitro* inhibition of both promastigotes and amastigotes when combined with miltefosine. Increased *in vivo* leishmanicidal activity, along with increased production of toxic reactive oxygen and nitrogen metabolites, was also caused by combination therapy³⁷. The bioavailability of curcumin was enhanced when nanoparticles were administered. At the subcurative dose, miltefosine leishmanicidal effect was supported by poly (lactide-co-glycolide) loaded with curcumin (nanocurcumin) through immunomodulation. No hepatotoxic or nephrotoxic effects were observed with nanocurcumin monotherapy or in combination with miltefosine³⁷.

The apoptotic pathway in *Leishmania* is still unknown; however, the proteins involved in apoptosis are also unknown. *Leishmania* lacks the mammalian key proteins involved in apoptosis³². In *L. major*, although LmjMCA (a cysteine peptidase) has different substrate specificities, it shares similarities with caspases. In a recent report, three pathways involved in apoptosis have been identified in *L. major*: the first pathway through which miltefosine mediated LmjMCA activation; the second pathway through which LmjMCA was inhibited by amphotericin B, curcumin and H₂O₂ and the third apoptotic pathway that was induced by pentamidine and considered to be independent of LmjMCA. A previous report also found that LmjMCA plays a role similar to caspases in both cell death and autophagy in *L. major*⁸. The cell death effector protein LmjHYD36 overexpression

was induced by curcumin in both the *L. major* wild-type strain and the LmjMCA-deficient strain⁴.

It was suggested that different apoptotic pathways in *Leishmania* should be involved. Non-caspase proteases, such as calpains, have been involved in *Leishmania* cell death⁷; however, cathepsin B-like enzyme LmjCPC has also been detected in different domains¹⁸. In a previous study, trans-dibenzalacetone (DBA), a synthetic curcumin analog demonstrated leishmanicidal efficacy against *L. donovani* amastigotes. In addition to cell cycle arrest at the G0/G1 phase, it exhibited antiproliferative effects with increased concentration of cytosolic calcium and dissipation of mitochondrial membrane potential. DBA also induced inhibition of the trypanothione/trypanothione reductase (TR) system of *Leishmania* cells¹⁰. Using curcumin analog DBA, *L. donovani* enters the late apoptotic stage without passing through the early apoptotic phase; however, in our study curcumin showed both early and late apoptosis in *L. major* and this could be explained by the different modes of action of the two different therapeutic molecules or by the different apoptotic pathways of the different *Leishmania* strains. Curcumin and miltefosine increased *L. major* apoptosis by overexpressing LmjF.22.0600, a potential acetyltransferase that is conserved between different *Leishmania* species³.

In a previous research, the photodynamic activity of curcumin on *L. major* and *Leishmania braziliensis* promastigotes was examined. The trypan blue test showed that curcumin photodynamic treatment had a significant effect on the viability and morphology of parasites. The rise in curcumin concentration in both the nucleus and kinetoplast of *Leishmania* clarified the increased mitochondrial activity, which suggested apoptosis³¹. In addition, curcumin is a potent inhibitor of promastigote proliferation by delaying the S-phase cell cycle. The inhibition was very sharp after just 24h of treatment. It has been previously shown that curcumin induced cell cycle arrest at the G2/M phase in *L. donovani* promastigotes¹³. Curcumin may inhibit certain biological reactions in mammalian cells, for example, we have previously shown that curcumin inhibited cell proliferation and triggered the G2/M phase of cell-cycle arrest in medulloblastoma cells¹⁷. Curcumin has also been reported to interrupt cell cycle progression in colon cancer cells²³.

Collectively, these data show that curcumin has potent leishmanicidal and antiproliferative properties against *L. major* promastigotes. It triggers cell death mainly by apoptosis and thus warrants further investigations of its potential use as a chemotherapeutic agent against cutaneous leishmaniasis. Future studies on intracellular amastigotes and animal models are recommended. Molecular studies should be conducted to further investigate the apoptotic pathway triggered by this molecule.

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgements

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References

- Aggarwal BB, Kumar A, Bharti AC. Anticancer potential of curcumin: preclinical and clinical studies. *Anticancer Res.* 2003;23:363–98.
- Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, Jannin J, den Boer M, Team MW. Leishmaniasis worldwide and global estimates of its incidence. *PLoS ONE.* 2012;7:e35671.
- Basmaciyan L, Azas N, Casanova M. A potential acetyltransferase involved in *Leishmania major* metacaspase-dependent cell death. *Parasit Vectors.* 2019;12:266.
- Basmaciyan L, Jacquet P, Azas N, Casanova M. A novel hydrolase with a pro-death activity from the protozoan parasite *Leishmania major*. *Cell Death Discov.* 2019;5:99.
- Bero J, Hannaert V, Chataigné G, Hérent MF, Quetin-Leclercq J. *In vitro* antitrypanosomal and antileishmanial activity of plants used in Benin in traditional medicine and bio-guided fractionation of the most active extract. *J Ethnopharmacol.* 2011;137:998–1002.
- Bornfreund E, Babich H, Martin-Alguacil N. Comparison of two *in vitro* cytotoxicity assays: the neutral red (NR) and tetrazolium (MTT) tests. *Toxicol In Vitro.* 1988;2:1–6.
- Branquinho MH, Marinho FA, Sengenito LS, Oliveira SS, Gonçalves KC, Ennes-Vidal V, d'Ávila-Levy CM, Santos AL. Calpains: potential targets for alternative chemotherapeutic intervention against human pathogenic trypanosomatids. *Curr Med Chem.* 2013;20:3174–85.
- Casanova M, Gonzalez IJ, Sprissler C, Zalila H, Dacher M, Basmaciyan L, Späth GF, Azas N, Fasel N. Implication of different domains of the *Leishmania major* metacaspase in cell death and autophagy. *Cell Death Dis.* 2015;6:e1933.
- Castaño JC, Alejandra María Giraldo AM. Antiparasitic phytotherapy perspectives, scope and current development. *Infect.* 2019;23:189–204.
- Chauhan IS, Rao GS, Shankar J, Chauhan LKS, Kapadia GJ, Singh N. Chemoprevention of Leishmaniasis: in-vitro antiparasitic activity of dibenzalacetone, a synthetic curcumin analog leads to apoptotic cell death in *Leishmania donovani*. *Parasitol Int.* 2018;67:627–36.
- Crissman HA, Hirons GT. Staining of DNA in live and fixed cells. *Methods Cell Biol.* 1994;41:196–209.
- Cui L, Miao J, Cui L. Cytotoxic effect of curcumin on malaria parasite *Plasmodium falciparum*: inhibition of histone acetylation and generation of reactive oxygen species. *Antimicrob Agents Chemother.* 2007;51:488–94.
- Das R, Roy A, Dutta N, Majumder HK. Reactive oxygen species and imbalance of calcium homeostasis contributes to curcumin-induced programmed cell death in *Leishmania donovani*. *Apoptosis.* 2008;13:867–82.
- Devassy J, Nwachukwu I, Jones P. Curcumin and cancer: barriers to obtaining a health claim. *Nutr Rev.* 2015;73:155–65.
- Dujardin JC, Campino L, Canavate C, Dedet JP, Gradoni L, Soteriadou K, Maz-eris A, Ozbek Y, Boelaert M. Spread of vector-borne diseases and neglect of Leishmaniasis Europe. *Emerg Infect Dis.* 2008;14:1013–8.
- Elamin MH, Al-Maliki SS. Leishmanicidal and apoptotic activities of oleuropein on *Leishmania major*. *Int J Clin Pharmacol Ther.* 2014;52:880–8.
- Elamin MH, Shinwari Z, Hendrayani F, Al-Hindi H, Al-Shail E, Khafaga Y, Al-Kofide A, Aboussekhra A. Curcumin inhibits the sonic hedgehog signaling pathway and triggers apoptosis in medulloblastoma cells. *Mol Carcinog.* 2010;49:302–14.
- El-Fadili AK, Zangger H, Desponds C, Gonzalez IJ, Zalila H, Schaff C, Ives A, Masina S, Motttram JC, Fasel N. Cathepsin B-like and cell death in the unicellular human pathogen *Leishmania*. *Cell Death Dis.* 2010;1:e71.
- European Food Safety Authority. Refined exposure assessment for curcumin (E 100). *EFSA J.* 2014;12:3876.
- Fuertes MA, Nguema PA, Castilla J, Alonso C, Pérez JM. Anticancer compounds as leishmanicidal drugs: challenges in chemotherapy and future perspectives. *Curr Med Chem.* 2008;15:433–9.
- Goel A, Kunnumakkara KB, Aggarwal BB. Curcumin as "Curcumin": from kitchen to clinic. *Biochem Pharmacol.* 2008;75:787–809.
- Koide T, Nose M, Ogihara Y, Yabu Y, Ohta N. Leishmanicidal effect of curcumin *in vitro*. *Biol Pharm Bull.* 2002;25:131–3.
- Lim TG, Lee SY, Huang Z, Lim do Y, Chen H, Jung SK, Bode AM, Lee KW, Dong Z. Curcumin suppresses proliferation of colon cancer cells by targeting CDK2. *Cancer Prev Res.* 2014;7:466–74.
- Liu Y, Peterson D, Kimura H, Schubert D. Mechanism of cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction. *J Neurochem.* 1997;69:581–93.
- Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods.* 1983;65:55–63.
- Nose M, Koide T, Ogihara Y, Yabu Y, Ohta N. Trypanocidal effects of curcumin *in vitro*. *Biol Pharm Bull.* 1998;21:643–5.
- Pagliano P, Carannante N, Rossi M. Visceral leishmaniasis in pregnancy: a case series and a systematic review of the literature. *J Antimicrob Chemother.* 2005;55:229–33.
- Passero LF, Bonfim-Melo A, Corbett CEP, Laurenti MD, Toyama MH, de Toyama DO, Romoff P, Fávero OA, dos Grecco SS, Zalewsky CA, Lago JH. Anti-leishmanial effects of purified compounds from aerial parts of *Baccharis uncinella* C. (Asteraceae). *Parasitol Res.* 2011;108:529–36.
- Pérez-Arriaga L, Mendoza-Magaña ML, Cortés-Zárate R, Corona-Rivera A, Bobadilla-Morales L, Troyo-Sanromán R, Ramírez-Herrera MA. Cytotoxic effect of curcumin on *Giardia lamblia* trophozoites. *Acta Trop.* 2006;98:152–61.
- Pijpers J, den Boer ML, Essink DR, Ritmeijer K. The safety and efficacy of miltefosine in the long-term treatment of post-kala-azar dermal leishmaniasis in South Asia – a review and meta-analysis. *PLoS Negl Trop Dis.* 2019;13:e0007173.
- Pinto GJ, Fontana LC, de Oliveira MA, Kurachi C, Raniero LJ, Ferreira-Strixino J. *In vitro* evaluation of photodynamic therapy using curcumin on *Leishmania major* and *Leishmania braziliensis*. *Lasers Med Sci.* 2016;31:883–90.
- Proto WR, Coombs GH, Motttram JC. Cell death in parasitic protozoa: regulated or incidental? *Nat Rev Microbiol.* 2013;11:58–66.
- Reddy RC, Vatsala PG, Keshamouni VG, Padmanaban G, Rangarajan PN. Curcumin for malaria therapy. *Biochem Biophys Res Commun.* 2005;326:472–4.
- Saleheen D, Ali SA, Ashfaq K, Siddiqui AA, Agha A, Yasinzaï MM. Latent activity of curcumin against Leishmaniasis *in vitro*. *Biol Pharm Bull.* 2002;25:386–9.
- Sen N, Das BB, Ganguly A, Mukherjee T, Tripathi G, Bandyopadhyay S, Rakshit S. Camptothecin induced mitochondrial dysfunction leading to programmed cell death in unicellular hemoflagellate *Leishmania donovani*. *Cell Death Differ.* 2004;11:924–36.
- Strimpakos AS, Sharma RA. Curcumin: preventive and therapeutic properties in laboratory studies and clinical trials. *Antioxid Redox Signal.* 2008;10:511–45.
- Tiwari B, Pahuja R, Kumar P, Rath SK, Gupta KC, Goyal N. Nanotized curcumin and miltefosine, a potential combination for

- treatment of experimental visceral leishmaniasis. *Antimicrob Agents Chemother.* 2017;61:e01169–1216.
38. Vermes I, Haanen C, Steffens-Nakken H, Reutelingsperger C. A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labeled Annexin V. *J Immunol Methods.* 1995;184:39–51.
39. World Health Organisation (WHO). Cutaneous leishmaniasis. Why are you neglecting me?; 2007. Disponible en: <https://apps.who.int/iris/handle/10665/69916>.