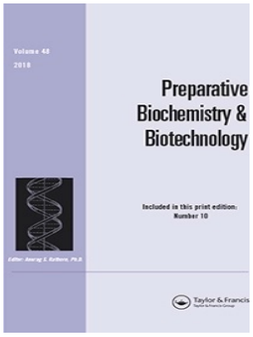

Taylor & Francis
Taylor & Francis Group

Preparative Biochemistry & Biotechnology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/lpbb20

A novel delivery of curcumin by the efficient nanoliposomal approach against *Leishmania major*

Ali Fattahi Bafghi, Bibi Fatemeh Haghirosadat, Fatemeh Yazdian, Farzaneh Mirzaei, Mehrab Pourmadadi, Fahimeh Pournasir, Mahdie Hemati & Soheila Pournasir

To cite this article: Ali Fattahi Bafghi, Bibi Fatemeh Haghirosadat, Fatemeh Yazdian, Farzaneh Mirzaei, Mehrab Pourmadadi, Fahimeh Pournasir, Mahdie Hemati & Soheila Pournasir (2021): A novel delivery of curcumin by the efficient nanoliposomal approach against *Leishmania major*, Preparative Biochemistry & Biotechnology, DOI: 10.1080/10826068.2021.1885045

To link to this article: https://doi.org/10.1080/10826068.2021.1885045

Published online: 01 Jun 2021.

PREPARATIVE BIOCHEMISTRY & BIOTECHNOLOGY
https://doi.org/10.1080/10826068.2021.1885045

Taylor & Francis
Taylor & Francis Group

A novel delivery of curcumin by the efficient nanoliposomal approach against *Leishmania major*

Ali Fattahi Bafghi[a], Bibi Fatemeh Haghirosadat[b], Fatemeh Yazdian[c], Farzaneh Mirzaei[a], Mehrab Pourmadadi[c], Fahimeh Pournasir[a], Mahdie Hemati[d], and Soheila Pournasir[a]

[a]Department of Medical Parasitology and Mycology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran; [b]Department of Advanced Medical Sciences and Technologies, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran; [c]Department of Life Science Engineering, Faculty of New Sciences and Technology, University of Tehran, Tehran, Iran; [d]Department of Clinical Biochemistry, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

ABSTRACT

Several side effects and drug resistance accompany the current therapies for Leishmaniasis. Nanoliposomal curcumin is applied as a new therapy approach instead of current therapy. In this study, nanoliposomal curcumin was prepared using thin-film hydration method and characterized based on encapsulation efficiency, size, and zeta potential. Curcumin was successfully loaded into nanoliposomes with an encapsulation efficiency of 92%. The surface charge of the nanoparticle was neutral, and the size of nanoparticle was 176.5 nm. Nanoliposomal curcumin is in spherical shape without any agglomeration. Cell viability assay was performed on HFF cell line to show biocompatibility of liposome nanoparticles. Anti-Leishmanial effect of different concentrations of liposomal curcumin (0.05–30 μg mL^{-1}) and amphotericin B (25 μg mL^{-1}) were studied on *Leishmania major* [MRHO/IR/75/ER] at various hours (24, 48, and 72) using hemocytometer technique. Nanoliposomal curcumin inhibitory concentration (IC50) at hours 24, 48, and 72 were 6.41, 3.8, and 2.33 μg mL^{-1}, respectively. As prepared nanoliposomal curcumin showed a significant anti-leishmanial effect and induced a better and more tangible effect on the survival of *L. major* promastigotes and could be suitable candidates for further investigations.

KEYWORDS

Curcumin; drug delivery; *in vitro*; *Leishmania*; nanoliposome

Introduction

Leishmaniasis is considered as a severe infection of the World Health Organization.[1] The treatment of Leishmaniasis is difficult because of the intramacrophagic location of the infectious form. In the absence of a vaccine, there is an urgent need for effective drugs to replace or supplement those in current use.[2,3] leishmaniasis is a complex of prevalent parasitic infections induced by a kind of obligatory intracellular protozoa of blood and tissues from the Tryponosomatidae of the *Leishmania* genus that cause numerous clinical manifestations, such as visceral, mucocutaneous, and cutaneous leishmaniasis.[1,4] Leishmaniasis is a disease that occurs at a rate of 12–15 million/year of people all around the world.[4] Development resistance and cardio and renal cytotoxicity restricted current drug for Leishmaniasis therapy are including Glucantime and Pentostam.[5] Herbal medicines and natural formulations are new candidates for treating Leishmaniasis. Curcumin belongs to the Zingiberaceae family and is one of the main ingredients of the rhizome of Curcuma longa with antioxidant, antiparasitic, antifungal, antibacterial, antiviral, anti-inflammatory, and antiproliferative properties.[6–8] The *in vitro* leishmanicidal activity of curcumin against *L. major* has been addressed in previous studies.[9] Since curcumin is a hydrophobic compound, it has limited bioavailability and low absorption. These properties have limited the applicability of curcumin for the treatment of *Leishmania*. The drug delivery systems, such as polysaccharides, liposomes, nanoparticles, niosome, etc., have facilitated an efficient way to increase the solubility of hydrophobic molecules and their bioavailability.[10] Thanks to the recent drug delivery systems, the drug release rate along with the drug concentration at the site of *Leishmania* infection has been markedly improved, and drug dosage required for parasitic infection has substantially reduced.[11,12] Liposomes are artificial vesicles with spherical-like shapes commonly made of phospholipids and cholesterol. The enhanced biocompatibility, stability, ease of scaling up, and ability to load various molecules, have made liposomes a bona fide candidate to be employed for drug delivery system in a broad range of treatment protocols. Delivery of curcumin using liposomal nanoparticles causes to increase the solubility of curcumin in plasma and skin tissue as well as decreasing drug release rate, which subsequently improves the therapeutic impact of curcumin (Figure 1).[13] In fact, nanoliposome provide a valuable feature to penetrate easily into parasite tissue and can eliminate parasitic infections without affecting the

CONTACT Ali Fattahi Bafghi afbafghi@gmail.com Department of Medical Parasitology and Mycology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

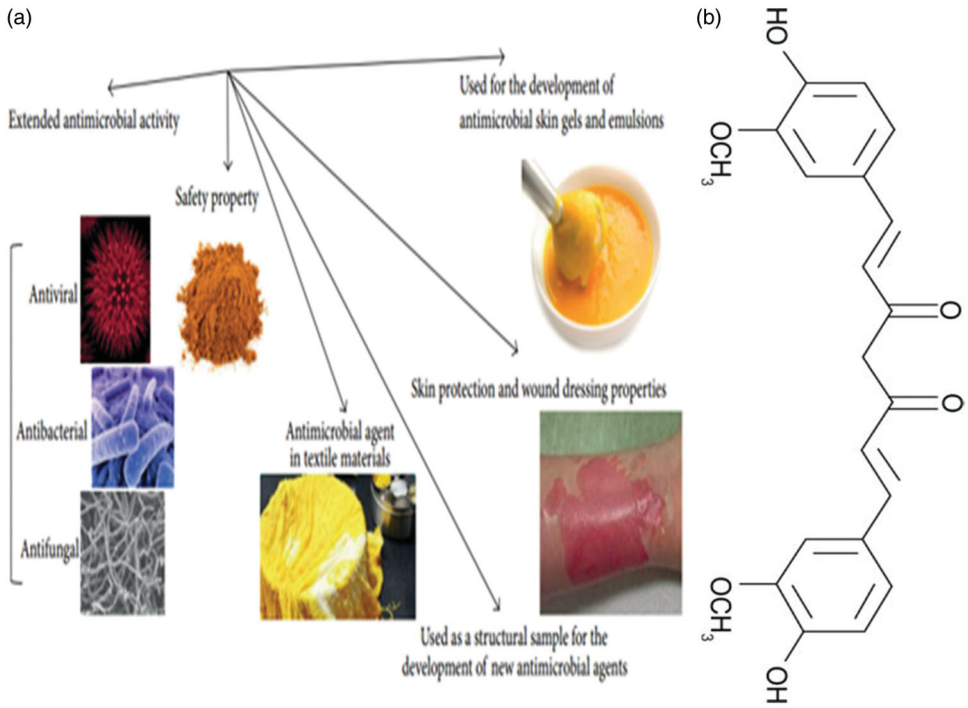

Figure 1. (a) An importance of curcumin in antimicrobial studies, (b) chemical structure of curcumin.[1]

normal tissues. Curcumin compounds nanoparticles have been used for different medical applications caused by their small nanoscale sizes and their high surface-to-volume ratios that allow more active sites for interacting with biological molecules such as cancer cell, microbes and other bioactive entities.[9,14] Thus, in this study, we successfully synthesized, characterized, and established a new approach for the efficient transfer of novel nanoliposomes containing curcumin against *Leishmania major*.

Materials and methods

Chemicals and biological sample

Leishmania major [MRHO/IR/75/ER] and HFF (Human Primary Foreskin Fibroblast Cells) were provided by Pasteur Institute of Iran, Tehran. MTT and DMSO (Dimethyl Sulfoxide) were purchased from Sigma-Aldrich (St. Louis, MO). DMEM and RPMI-1640 cell culture were obtained from Gibco Invitrogen (GmbH, Karlsruhe, Germany). Heat-inactivated fetal bovine serum (FBS), fetal calf serum (FCS), and penicillin/streptomycin were supplied by Invitrogen (Carlsbad, CA). Soy phosphatidylcholine was obtained from Lipoid (Lipoid GmbH, Germany). Cholesterol and curcumin are also supplied by Sigma (Sigma-Aldrich). All other chemical reagents were of the purest grade available (>99%).

Preparation of nanoliposomal curcumin

Liposomal curcumin was prepared using thin-film hydration method.[15] In brief, 0.0934 g of soy phosphatidylcholine, 0.0196 g of cholesterol, and 0.003 g of curcumin were dissolved in 3–4 ml of chloroform which then evaporated under vacuum condition at 45° C using a rotary evaporator (Heidolph, Germany). Dried film was hydrated with 5.5 ml of sterilized distilled water while rotating for 30 min at 55 °C to perform spherical vesicles. Liposome particles were subsequently reduced using both microtip probe sonicator (E–Chrome Tech Co, Taiwan) for 10 min and bath ultrasonic (Elmasonic S, Germany) at 45 °C for 1 h. The vesicles were homogenized, and their size was further reduced using 0.22 µm filtration. The untrapped drug was removed by dialysis method using cellulose tube (cut-off 12–14 kDa).

Physical characterization of liposomal vesicles

The size, polydispersity index (PDI) and zeta potential of the liposomal curcumin particles were measured by light scattering (Zeta-Sizer instrument, DLS, Malvern Zetasizer Nano-ZS, and Worcestershire, UK). Samples were diluted in water, and all the measurements were performed in a scattering angle of 90°. Surface morphology, the approximate size of particles, homogeneity of samples, and boundary between particles were studied using SEM (KYKYEM3200-30KV, China).

Encapsulation efficiency

At first, the untrapped drug was removed by dialysis method using cellulose tube (cut-off 12–14 kDa). Then to break down the liposomal formulations and dissolve the curcumin, the liposomal curcumin diluted in isopropanol in 1:20 volume ratio and determined using a spectrophotometer (model T80+, PG Instruments, UK) at 430 nm.

In vitro drug release

Release of curcumin from nanoliposome was investigated using dialysis method after immersing dialysis membrane containing liposomal curcumin in a phosphate buffer saline at 37 °C and pH 7.4 under gentle shacking condition. At predetermined time interval until 72 hr, 1 ml of surrounding phosphate-buffered saline (PBS) solution was withdrawn and replaced with fresh buffer. The released drug was determined using a spectrophotometer at 430 nm wavelength and by comparing with a standard curve of different concentrations of curcumin in isopropanol.

Mathematical modeling of drug delivery

Mathematical modeling of drug delivery as reported:[16]

Drug released from the nanocarrier were evaluated using the equation as below:

$$\text{Amount of release} = M_t/M_f \quad (1)$$

M_t and M_f are the amounts of drug at t and the final amount of drug released, respectively.

Zero-order rate correlation is as below:

$$Q_t = Q_0 + K_0 t \quad (2)$$

Where Q_t and Q_0 are the remaining drugs at t and the initial value of the drug, respectively.

For first-order rate relation:

$$\text{Log } C = \log C_0 - Kt/2.303 \quad (3)$$

K is the first-order release constant.

Higuchi's model is as below:

$$Q = K_H t^{1/2}$$

Q is the value of drug released in t per surface, and K_H is constant.

Cell viability and anti-Leishmanial activity of nanoliposomal curcumin

The biocompatibility of prepared liposome against skin fibroblast cell line (HFF) was confirmed by measuring relative cell viability using MTT assay. In brief, HFF cells were seeded onto 96-well plates at a density of 10^4 cells/well and cultivated for one night in growth medium before the administration of blank liposome. On the following day, when cells reached 80%, confluences cells were treated with blank liposome at a concentration of 100 and 1000 µg mL^{-1}. After 48 hr of incubation in 5% CO_2, at 37 °C, the cells were washed twice with PBS and growth medium was replaced with MTT solution diluted in PBS, and the cells were then incubated for further 4 hr. Following that, the medium and MTT solution was drained up with DMSO to dissolve the formazan crystals. The light absorbance of samples was then measured with an EPOCH Microplate Spectrophotometer (synergy HTX, Bio Tek) at 570 and 630 nm. The cells without any sample were considered as control.

For the survey of anti-Leishmanial activity of nanoliposomal curcumin, *L. major* [MRHO/IR/75/ER] was cultured in Novy-Nicolle-Mac Neal (NNN) medium which was subsequently were adopted to RPMI-1640 medium supplemented with 20% FCS, penicillin (100 U mL^{-1}), streptomycin (100 µg mL^{-1}), and glutamine at 25 °C. Approximately 1×10^6promastigotes, which were in their early stationary phase, were transferred to each microtube. Then nanoliposomal curcumin added in certain microtubes with final concentrations of 0.05, 0.1, 0.2, 0.4, 0.81, 1.62, 3.25, 7.5, 15, and 30 µg mL^{-1}.

Moreover, two control groups, including a negative control containing PBS and the parasite as well as a positive control with Amphotericin B (25 µg mL^{-1}) and the parasite, were considered. After 24, 48, and 72 hr, the final number of viable parasites (with 0.4% trypan blue were considered as viable ones) was counted using a hemocytometer under light microscopy. The value of 50% inhibitory concentration (IC50) was calculated, and the graph was plotted using SigmaPlot™13 (Systat Software Inc). The percentage of growth inhibition (% GI) that used for calculated IC50 was calculated with respect to the growth control as follows:[17]

$$\%\ \text{GI} = \left(1 - \frac{\text{GR}_{\text{extract}}}{\text{GR}_{\text{control}}}\right)100$$

The experiments were accomplished in triplicate and repeated at least two times and single-blind conditions.

Statistical analysis

All experiments were carried out in triplicate, and the IC50 values were calculated with SigmaPlot™13 (Systat Software Inc). *T*-test analysis, ANOVA was used to compare the growth value in one group with the control group. Statistical difference less than 5% (p-value <0.05) was considered as a statistical significance.

Results

Characterization of liposomal curcumin

To measure the size, PDI, zeta potential, and morphological of nanoliposomal curcumin, As shown in Figure 2, the size of nanoliposome-loaded curcumin is 176.5 nm. The PDI of curcumin-loaded nanoliposome is 0.187 is less than 0.30, which means no aggregation occurred. As indicated in Figure 3, the zeta potential of liposome is approximately −23.25 mV and significantly change after Curcumin encapsulation to 34.99 mv. The surface morphology of nanoliposome was studied using Scanning Electron Microscopy (SEM) imaging, and the image has been shown in Figure 4. Prepared liposomal curcumin is in a spherical shape with well-identified boundary. There is no obvious change in the shape of nanoparticles, and the approximate size of nanoparticles is less than 200 nm.

Encapsulation efficiency

The encapsulation efficiency of curcumin into nanoparticle was indirectly determined using dissolving liposome

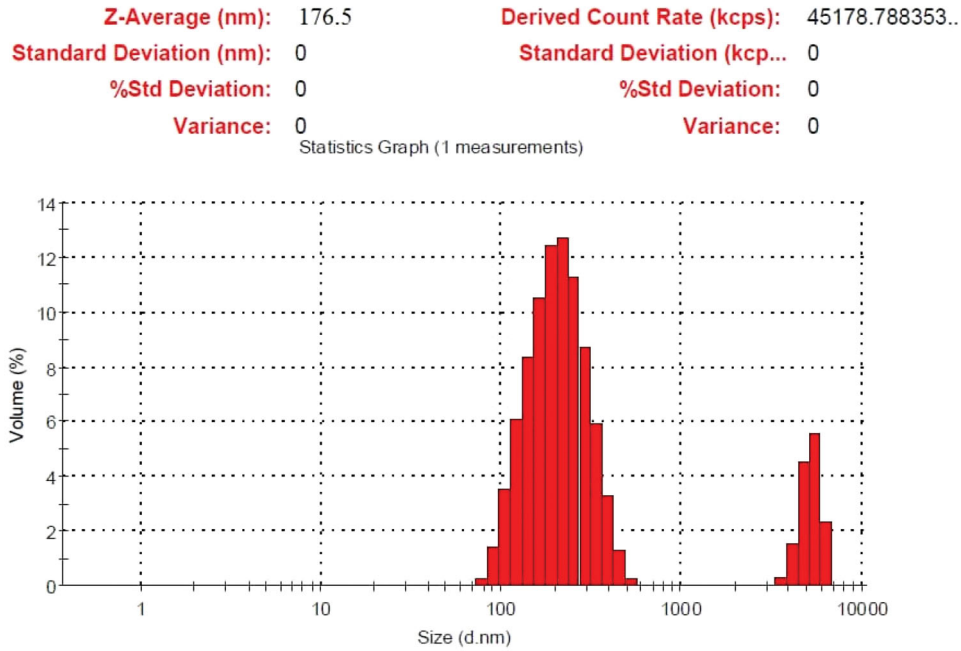

Figure 2. Particle size of nanoliposome after loading curcumin.

suspension in isopropanol to break lipid membrane, and leak entrapped Curcumin into Isopropanol following by spectrophotometry. The encapsulation efficiency of Curcumin into liposome was determined to be 92%.

Drug release

In vitro release profile of curcumin in PBS buffer from prepared liposome vesicles was monitored during 3 days in PBS buffer PH = 7.4 at 37 °C. As illustrated in Figure 5, about 40% of the Curcumin is totally released within the 3 days of incubation nanoparticles. Curcumin release occurred in a sustained manner with an initial burst. The results obtained from the drug release indicated that the release rate of curcumin (at the concentrations of 30 µg mL^{-1}) was 39.3 ± 6.2% after 72 hr, led to the complete eradication (approximately 100%) of *Leishmania major*. It could be inferred that curcumin-containing nanoliposomes show higher toxicity against parasite cells when used at lower doses.

Modeling of drug release

Table 1 shows the correlation coefficient (*R*-square) evaluated for nanocarrier formulation. The results revealed that the curcumin from the nanocarrier film is most fitted to Higuchi's correlation due to higher R^2. Also, Figure 6a shows the Higuchi release kinetics for curcumin. Figure 6b confirms the zero-order release kinetics for curcumin as well. The first-order model of Curcumin release was shown in Figure 6c.

Cell viability of blank nanoliposome

The biocompatibility of prepared liposome against skin fibroblast cells was investigated using MTT assay after 48 h treating cells with blank liposome at 100 and 1000 µg mL^{-1}concentration. According to the results demonstrated in Figure 7, the prepared liposome does not cause any toxicity in both concentrations. On the other hand, the prepared formulation might reduce the side effect of current anti-Leishmania drugs.

The anti-Leishmania activity of curcuminloaded nanoliposome

The results of anti-leishmanial activity of various concentrations of nanoliposome curcumin is shown in Figure 8. The results demonstrated that the IC50 values of curcumin-containing nanoliposomes were estimated as 6.41, 3.8, and 2.33 µg mL^{-1} when incubated with parasite cells at 24, 48, 72 hr, respectively. The mean percentage of viability of *Leishmania major* promastigotes after 24, 48 and 72 hr in various nanoliposomal curcumin concentrations (0.05–30 µg mL^{-1}) showed statistically significant difference compared to the negative control ($p < 0.05$).

Discussion

Currently using the drug for Leishmaniasis include glucantime and pentamidine, is accompanied by high costs, toxicity, long-term treatment period, minor impact, and drug resistance.[17,18] Despite all the attempts made to prevent the disease, the prevalence of this disease is increasing in developing countries. So far, there is no definitely effective drug available for the treatment of this infection. Hence, the greatest necessity at the present time is the preparation of a

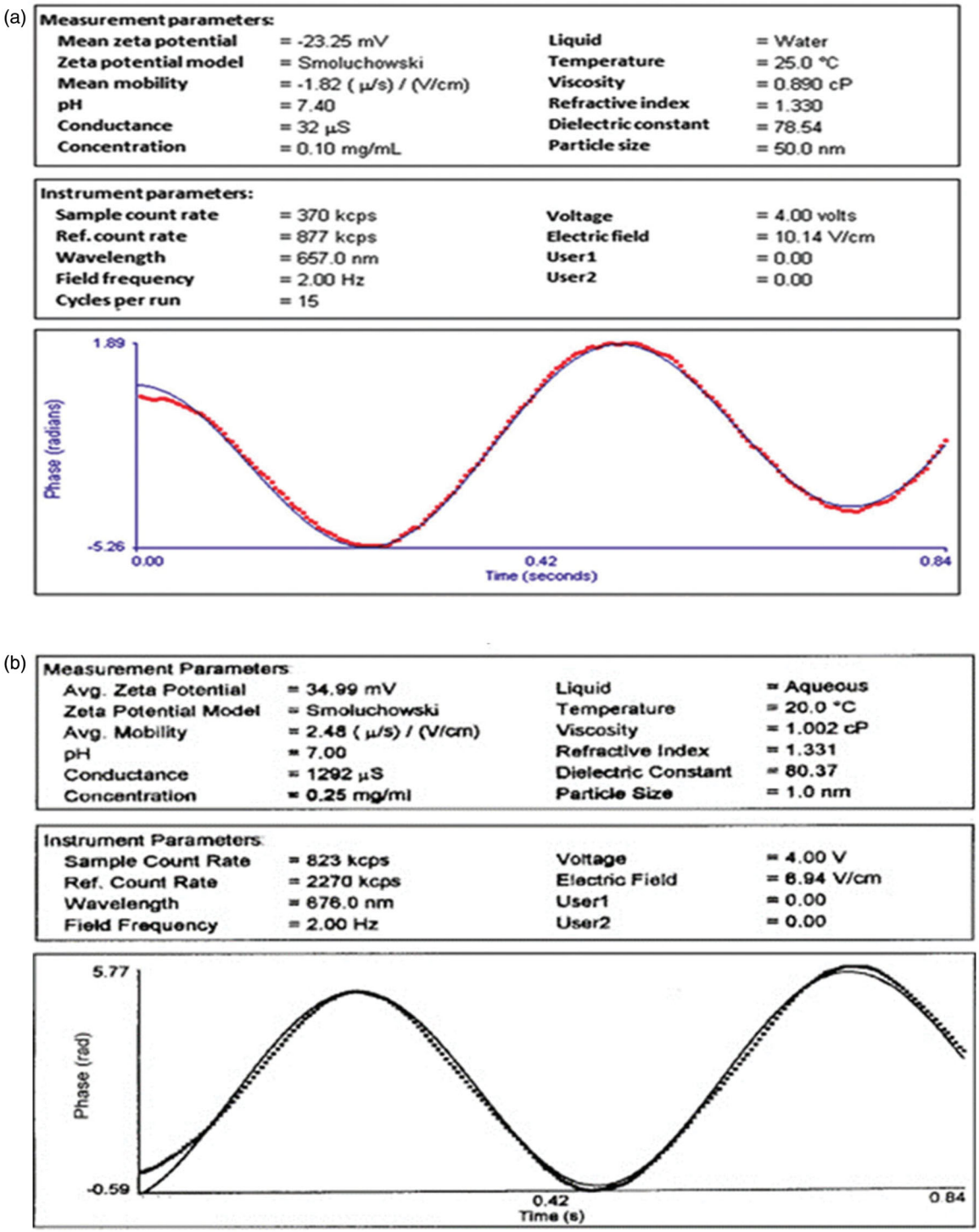

Figure 3. Zeta potential of nanoliposome (a) before drug loading and (b) after drug loading.

suitable, efficacious, definite, and cheap drug. Curcumin as herbal medicines have extensive applications in medicine as anticancer, antioxidant, antimicrobial, antiparasitic, antivirus, and antidiabetic agents, and their effects are related to the immune system.[19] To improve its efficacy, rapid absorption of the drug, and maximizing its bioavailability in the target tissue, reducing its toxicity, and decreasing the administered dose, drug delivery vehicles at the nano scale-like liposome are used as innovative drug delivery systems.[20] Nanoliposomal curcumin was exposed to stationary phases of the parasite at 24, 48, and 72 hr that IC50 for these times were 6.41, 3.8, and 2.33 µg.mL^{-}1, respectively. In general, curcumin nanoparticles are effective on the survival of parasitic promastigotes in rural villagers and reduce the viability of parasite promastigotes, which is more effective than Amphotericin B. Nanoliposomal curcumin are effective in preventing the growth of the *Leishmania* parasite, and in the past, it has been used to treat curcumin or with other compounds against the parasite, and the results are indicative of its positive effect. As Brajenddra Tiwar

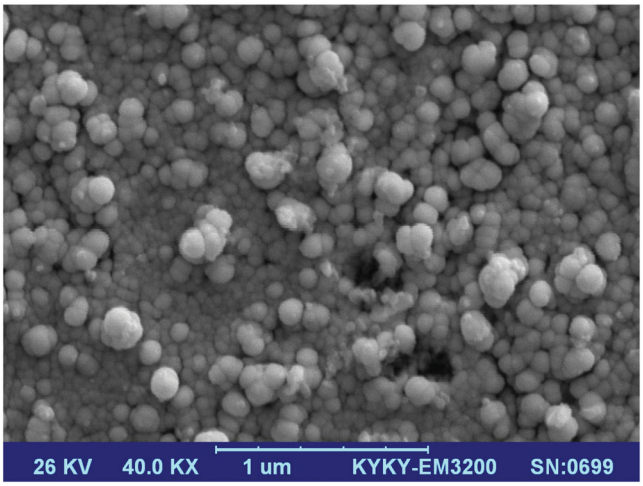

Figure 4. Surface morphology imaging of nanoliposomal carrier of curcumin.

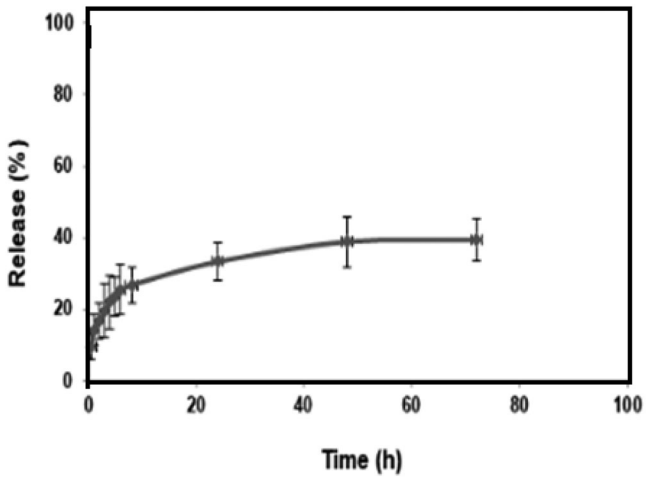

Figure 5. Drug release profile of curcumin during 3 days in PBS buffer (pH 7.4) at 37 °C.

Table 1. Data of release kinetic of curcumin from the nanocarrier.

Drug	Zero order	First order	Higuchi model
Curcumin	0.8106	0.9400	0.9800

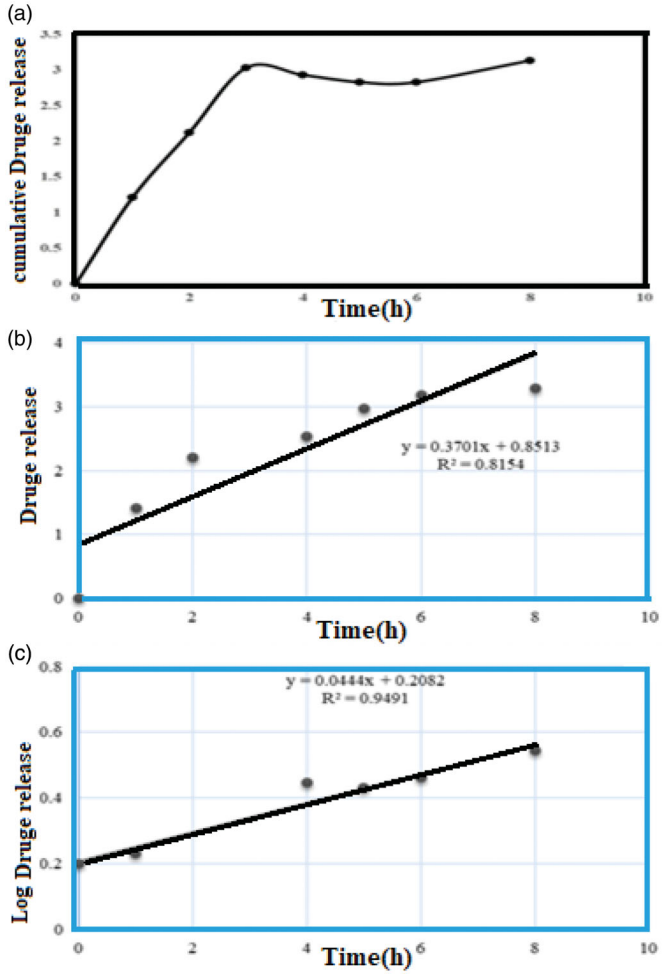

Figure 6. (a) Higuchi release kinetics for curcumin, (b) zero-order release kinetics for curcumin, (c) first-order release kinetics for curcumin.

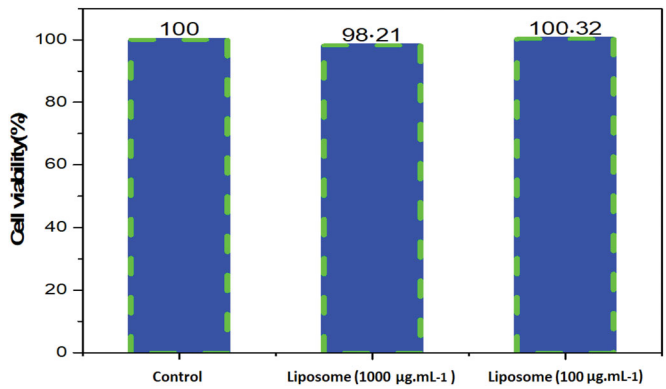

Figure 7. Cell viability of HFF cell line treated with blank liposome (1000 and 100 µg mL^{-1}) after 48-hr.

et al. in their study showed that the use of nanoliposomal curcumin as a supplementary adjunctive drug along with Miletphocin can be effective in the treatment and chemotherapy of visceral Leishmaniasis.[9] Moreover, Fouladv et al. found that the IC50 of curcumin, gallium curcumin, indium curcumin, and diacethyle curcumin on *L. major* were 38, 32, 26, and 52 µg mL^{-1}, respectively while it was 20 µg mL^{-1} for amphotericin B as control. Consequently, indium curcumin with IC50 = 26 µg mL^{-1} showed greater anti-leishmanial activity for inhibiting parasite growth compared to other derivatives.[21] Also, Samim et al. demonstrated that nano curcumin, compared to pentamidine and free curcumin in the animal model of visceral Leishmaniasis, manifested greater therapeutic effects than *in vitro* effects and provided a better method for treating the visceral leishmaniasis.[22] Koide et al. performed a study on the anti-leishmanial activity of curcumin (promastigotes of the *major* species) in SDM-79 culture medium for 24 hr. The total growth inhibition (TGI), growth inhibition$_{50}$ or GI_{50}, and lethal dose$_{50}$ or LD_{50} were estimated at 21, 38, and 12 µM, respectively.[23] Rasmussen et al. conducted another study on the effect of three phenol dike tone compounds, curcumin, demetoxy curcumin and bisdemetoxy curcumin, isolated from the rhizome of turmeric and found that these compounds had a moderate effect on *Plasmodium*

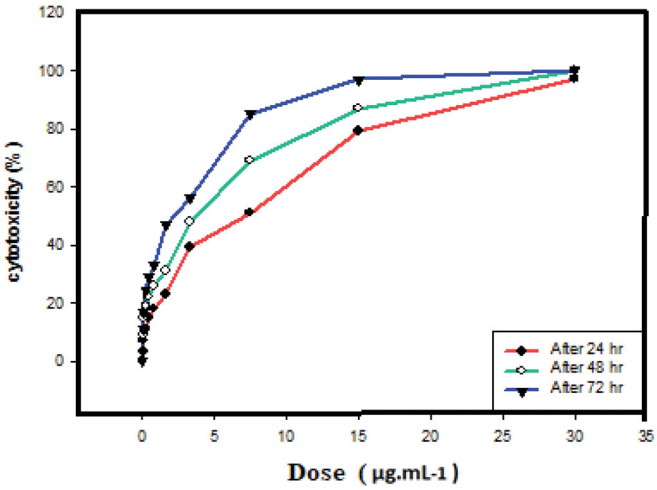

Figure 8. Effect of different concentration curcumin nanoparticles (CNPs) on survival of Leishmania major in the stationary phases.

falciparum (IC50 equal to 3.5, 4.2, and 3 µg mL^{-1}) and *Leishmania major* (IC50 equal to 7.8, 14.1, and 21.5 µg mL^{-1}) respectively.[24] The study by Saleheen et al. explored the effect of Curcumin on Promastigotes of three species of *Leishmania major*, *Leishmaniatropica*, and *Leishmaniainfantum*. The mean IC50 was reported as 5.3 µm, which was much smaller than the reference Pentamidine and was more effective than that.[25] Furthermore, Nose et al.[26] studied the effect of curcumin on trypanosomes and showed that curcumin properly affects trypanosomes. Moreover, many studies have been carried out on the effect of curcumin on prokaryotes (bacteria). Among them, the study by Rudrappa and Bais[27] revealed that curcumin is capable of controlling pathogenic factors and trigger (primer) genes of biofilm production in *Pseudomonas aeruginosa*. Furthermore, Singh et al.[28] assessed the antibacterial properties of curcumin and showed that curcumin is a powerful molecule in treating bacterial infections. Finally, the study by Rai et al.[29] demonstrated that curcumin could inhibit the growth of staphylococcus aureus.

Conclusion

Our findings confirm that nanoliposomal curcumin shows suitable anti-leishmanial activity compared to the current drugs as well as a less in vitro side effects. The viability rate of promastigotes of rural cutaneous Leishmaniasis, *Leishmania major*, treated by nanoliposomal curcuminis time-dependent in the stationary phase and leads to the kill of the parasite promastigotes.

Ethical approval

The experiments were confirmed by the Ethical Committee in Vice Chancellor of research of Error! Hyperlink reference not valid., (Ethical No: Ir.ssu.medicine.rec.1395.333).

Acknowledgments

This article is the result of the thesis No. 4781 approved by the School of Medicine, Yazd Shahid Sadoughi University of Medical Sciences. The authors of this article acknowledge their gratitude to Error! Hyperlink reference not valid. for its cooperation and financial support as well as colleagues who assisted us in the experimental procedure, especially Mrs Marziyeh Beigum Modares Sanavi, Mrs Nadernejad, and Mrs Zahra Esmaili.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This research received specific grant based on a thesis submitted No: 4781 in the School of Medicine, Yazd Error! Hyperlink reference not valid.. Yazd, Iran.

References

[1] Postigo, J. A. R. Leishmaniasis in the World Health Organization Eastern Mediterranean Region. *Int. J. Antimicrob. Agents* **2010**, *36*, S62–S65.

[2] Reithinger, R.; Dujardin, J.-C.; Louzir, H.; Pirmez, C.; Alexander, B.; Brooker, S. *Cutaneous Leishmaniasis. Lancet. Infect. Dis.* **2007**, *7*, 581–596.

[3] De Rycker, M.; Hallyburton, I.; Thomas, J.; Campbell, L.; Wyllie, S.; Joshi, D.; Cameron, S.; Gilbert, I. H.; Wyatt, P. G.; Frearson, J. A.; et al. Comparison of a High-Throughput High-Content Intracellular *Leishmania donovani* Assay with an Axenic Amastigote Assay. *Antimicrob Agents Chemother.* **2013**, *57*, 2913–2922.

[4] Bruni, N.; Stella, B.; Giraudo, L.; Della Pepa, C.; Gastaldi, D.; Dosio, F. Nanostructured Delivery Systems with Improved Leishmanicidal Activity: A Critical Review. *Int. J. Nanomed.* **2017**, *12*, 5289–5311.

[5] Mothana, R.; Al-Musayeib, N.; Matheeussen, A.; Cos, P.; Maes, L. Assessment of the in Vitro Antiprotozoal and Cytotoxic Potential of 20 Selected Medicinal Plants from the Island of Soqotra. *Molecules.* **2012**, *17*, 14349–14360.

[6] Kumar, A.; Chetia, H.; Sharma, S.; Kabiraj, D.; Talukdar, N. C.; Bora, U. Curcumin Resource Database. *Database* **2015**, *2015*, bav070.

[7] Moghadamtousi, S. Z.; Kadir, H. A.; Hassandarvish, P.; Tajik, H.; Abubakar, S.; Zandi, K. A Review on Antibacterial, Antiviral, and Antifungal Activity of Curcumin. *Biomed. Res. Int.* **2014**, *2014*, 186864.

[8] Aggarwal, B.; Deb, L.; Prasad, S. Curcumin Differs from Tetrahydrocurcumin for Molecular Targets, Signaling Pathways and Cellular Responses. *Molecules.* **2014**, *20*, 185–205.

[9] Tiwari, B.; Pahuja, R.; Kumar, P.; Rath, S. K.; Gupta, K. C.; Goyal, N. Nanotized Curcumin and Miltefosine, a Potential Combination for Treatment of Experimental Visceral Leishmaniasis. *Antimicrob. Agents Chemother.* **2017**, *61*, e01169–16.

[10] Zavareh, H. S.; Pourmadadi, M.; Moradi, A.; Yazdian, F.; Omidi, M. Chitosan/Carbon Quantum Dot/Aptamer Complex as a Potential Anticancer Drug Delivery System towards the Release of 5-Fluorouracil. *Int. J. Biol. Macromol.* **2020**, *165*, 1422–1430.

[11] Amaral, A. C. F.; Gomes, L. A.; Silva, J. R. d A.; Ferreira, J. L. P.; Ramos, A. d S.; Rosa, M. d S. S.; Vermelho, A. B.; Rodrigues, I. A. Liposomal Formulation of Turmerone-Rich

Hexane Fractions from *Curcuma longa* Enhances Their Antileishmanial Activity. *BioMed. Res. Int.* **2014**, *2014*, 1–8.

[12] Hemati, M.; Haghiralsadat, F.; Yazdian, F.; Jafari, F.; Moradi, A.; Malekpour-Dehkordi, Z. Development and Characterization of a Novel Cationic PEGylated Niosome-Encapsulated Forms of Doxorubicin, Quercetin and siRNA for the Treatment of Cancer by Using Combination Therapy. *Artif. Cells. Nanomed. Biotechnol.* **2019**, *47*, 1295–1311.

[13] Nylén, S.; Gautam, S. Immunological Perspectives of Leishmaniasis. *J. Glob. Infect. Dis.* **2010**, *2*, 135–146.

[14] Bland, S. D.; Venable, E. B.; McPherson, J. L.; Atkinson, R. L. Effects of Liposomal-Curcumin on Five Opportunistic Bacterial Strains Found in the Equine Hindgut – Preliminary Study. *J. Anim. Sci. Technol.* **2017**, *59*, 15.

[15] Varona, S.; Martín, A..; Cocero, M. J. Liposomal Incorporation of Lavandin Essential Oil by a Thin-Film Hydration Method and by Particles from Gas-Saturated Solutions. *Ind. Eng. Chem. Res.* **2011**, *50*, 2088–2097.

[16] Dash, S.; Murthy, P. N.; Nath, L.; Chowdhury, P. Kinetic Modeling on Drug Release from Controlled Drug Delivery Systems. *Acta Pol. Pharm.* **2010**, *67*, 217–223.

[17] Mirzaei, F.; Bafghi, A. F.; Mohaghegh, M. A.; Jaliani, H. Z.; Faridnia, R.; Kalani, H. In Vitro anti-leishmanial activity of *Satureja hortensis* and *Artemisia dracunculus* extracts on *Leishmania major* promastigotes. *J. Parasit. Dis.* **2016**, *40*, 1571–1574.

[18] Singh, O. P.; Singh, B.; Chakravarty, J.; Sundar, S. Current Challenges in Treatment Options for Visceral Leishmaniasis in India: A Public Health Perspective. *Infect. Dis. Poverty*. **2016**, *5*, 19.

[19] Yallapu, M. M.; Nagesh, P. K. B.; Jaggi, M.; Chauhan, S. C. Therapeutic Applications of Curcumin Nanoformulations. *AAPS J.* **2015**, *17*, 1341–1356.

[20] Marwah, H.; Garg, T.; Goyal, A. K.; Rath, G. Permeation Enhancer Strategies in Transdermal Drug Delivery. *Drug Deliv.* **2016**, *23*, 564–578.

[21] Fouladvand, M.; Barazesh, A.; Tahmasebi, R. Evaluation of in Vitro Antileishmanial Activity of Curcumin and Its Derivatives" Gallium Curcumin, Indium Curcumin and Diacethyle Curcumin. *Eur. Rev. Med. Pharmacol. Sci.* **2013**, *17*, 3306–3308.

[22] Samim, M.; Naqvi, S.; Arora, I.; Ahmad, F. J.; Maitra, A. Antileishmanial Activity of Nanocurcumin. *Ther. Deliv.* **2011**, *2*, 223–230.

[23] Koide, T.; Nose, M.; Ogihara, Y.; Yabu, Y.; Ohta, N. Leishmanicidal Effect of Curcumin *in Vitro*. *Biol. Pharm. Bull.* **2002**, *25*, 131–133.

[24] Rasmussen, H. B.; Christensen, S. B.; Kvist, L. P.; Karazmi, A. A Simple and Efficient Separation of the Curcumins, the Antiprotozoal Constituents of *Curcuma longa*. *Planta Med.* **2000**, *66*, 396–398.

[25] Saleheen, D.; Ali, S. A.; Ashfaq, K.; Siddiqui, A. A.; Agha, A.; Yasinzai, M. M. Latent Activity of Curcumin against Leishmaniasis *in Vitro*. *Biol. Pharm. Bull.* **2002**, *25*, 386–389.

[26] Nose, M.; Koide, T.; Ogihara, Y.; Yabu, Y.; Ohta, N. Trypanocidal Effects of Curcumin *in Vitro*. *Biol. Pharm. Bull.* **1998**, *21*, 643–645.

[27] Rudrappa, T.; Bais, H. Curcumin, a Known Phenolic from Curcuma Longa, Attenuates the Virulence of Pseudomonas aeruginosa PAO1 in Whole Plant and Animal Pathogenicity Models. *J. Agric. Food Chem.* **2008**, *56*, 1955–1962.

[28] Singh, R. K.; Rai, D.; Yadav, D.; Bhargava, A.; Balzarini, J.; De Clercq, E. Synthesis, Antibacterial and Antiviral Properties of Curcumin Bioconjugates Bearing Dipeptide, Fatty Acids and Folic Acid. *Eur. J. Med. Chem.* **2010**, *45*, 1078–1086.

[29] Rai, D.; Singh, J. K.; Roy, N.; Panda, D. Curcumin Inhibits FtsZ Assembly: An Attractive Mechanism for Its Antibacterial Activity. *Biochem. J.* **2008**, *410*, 147–155.