Gold-Conjugated Curcumin as a Novel Therapeutic Agent against Brain-Eating Amoebae

Mohammad Ridwane Mungroo, Ayaz Anwar, Naveed Ahmed Khan,* and Ruqaiyyah Siddiqui

ABSTRACT: Balamuthia mandrillaris and Naegleria fowleri are free-living amoebae that cause infection of the central nervous system, granulomatous amoebic encephalitis (GAE) and primary amoebic meningoencephalitis (PAM), respectively. The fact that mortality rates for cases of GAE and PAM are more than 95% indicates the need for new therapeutic agents against those amoebae. Considering that curcumin exhibits a wide range of biological properties and has shown efficacy against Acanthamoeba castellanii, we evaluated the amoebicidal properties of curcumin against N. fowleri and B. mandrillaris. Curcumin showed significant amoebicidal activities with an AC50 of 172 and 74 μM against B. mandrillaris and N. fowleri, respectively. Moreover, these compounds were also conjugated with gold nanoparticles to further increase their amoebicidal activities. After conjugation with gold nanoparticles, amoebicidal activities of the drugs were increased by up to 56 and 37% against B. mandrillaris and N. fowleri, respectively. These findings are remarkable and suggest that clinically available curcumin and our gold-conjugated curcumin nanoparticles hold promise in the improved treatment of fatal infections caused by brain-eating amoebae and should serve as a model in the rationale development of therapeutic interventions against other infections.

INTRODUCTION

Pathogenic free-living amoebae, including Naegleria fowleri and Balamuthia mandrillaris, are opportunistic protists that cause infection of the central nervous system (CNS). Infections with free-living amoebae almost always lead to death, indicating the lack of effective treatments against those virulent pathogens. N. fowleri is known to infect the CNS of young children and adults, causing primary amoebic meningoencephalitis (PAM), leading to a rapid onset of the disease and death within days. Amphotericin B, used in combination with rifampin and azole compounds, is the main treatment option but results in severe side effects, including nephrotoxicity. Like N. fowleri, B. mandrillaris also targets the CNS, causing the fatal disease termed granulomatous amoebic encephalitis (GAE). The mortality rate of patients suffering from GAE due to B. mandrillaris infections is very high, resulting in death in over 95% of the reported cases. Patients suffering from GAE caused by B. mandrillaris are usually treated with multidrug regimens, usually including miltefosine and pentamidine isethionate.

Curcumin is a hydrophobic polyphenol derived from the rhizome of the herb Curcuma longa. Curcumin, the active component of turmeric, has been traditionally used as an inflammatory agent in the treatment of several diseases. Human models and animal studies have demonstrated the extreme safety of curcumin. Curcumin expressed antioxidant activities in the rat brain as it inhibits lipid peroxidation at low concentrations. Micromolar concentrations of curcumin also resulted in the inhibition of superoxides and hydroxyl radicals, further confirming its antioxidant activities. Anti-inflammatory activities of curcumin in both chronic and acute models of inflammation have been reported. Antiviral activity of curcumin has been demonstrated in vivo in humans. Curcumin also possess antibacterial activities as demonstrated in a study where curcumin inhibited the growth of Helicobacter pylori. Antifungal and antiparasitic activities of curcumin have also been demonstrated, such as its inhibitory effects against Plasmodium falciparum at a low IC50 of 5 μM and its activities against Trichomonas vaginalis, Giardia lamblia, Toxoplasma gondii, and Cryptosporidium parvum.

Anticancer activities of curcumin at low micromolar concentrations have also been reported. Also clearly established are the myocardial...
Infarction protective, hepatoprotective, nephroprotective, thrombosis suppressing, and antirheumatic effects of curcumin. Curcumin has been shown to possess significant amoebicidal activities against _Acanthamoeba castellanii_. Curcumin can also affect the cysts of _A. castellanii_. However, the amoebicidal activities of curcumin against _N. fowleri_ and _B. mandrillaris_ are yet to be evaluated. We hypothesized that curcumin possesses antiamoebic activities against _N. fowleri_ and _B. mandrillaris_. Therefore, we evaluated the amoebicidal properties of curcumin against _N. fowleri_ and _B. mandrillaris_. We also conjugated curcumin with gold nanoparticles (AuNPs) to further enhance its amoebicidal properties.

**RESULTS AND DISCUSSION**

Pathogenic free-living amoebae, including _N. fowleri_ and _B. mandrillaris_, are opportunistic protists that cause infection of the CNS. The lack of effective treatments against those virulent pathogens is clearly indicated by the fact that infections with free-living amoebae almost always lead to death. _N. fowleri_ is known to infect the CNS, causing PAM, while _B. mandrillaris_ also targets the CNS, causing GAE.

Curcumin expressed antioxidant activities by inhibiting lipid peroxidation, superoxides, and hydroxyl radicals. Curcumin is known to exhibit antimicrobial, anti-inflammatory, and anticarcinogenic activities as well as therapeutic efficacy against various cardiovascular diseases, neurological disease, diabetes, and arthritis. Also clearly established are the myocardial infarction protective, hepatoprotective, nephroprotective, thrombosis suppressing, and antirheumatic effects of curcumin. The ability of curcumin to express neuroprotective abilities has been reported. This would indicate the ability of curcumin to penetrate the blood–brain barrier, and hence, it might be a treatment option for brain infections. Wound healing and tissue repair are intricate processes, involving inflammation, tissue remodeling, and granulation, that are enhanced by curcumin. This might help the patient in recovery from infections. Curcumin has been shown to possess significant amoebicidal activities against _A. castellanii_ trophozoites and cysts. Therefore, here, we evaluated the amoebicidal properties of curcumin against _N. fowleri_ and _B. mandrillaris_.

Curcumin was Successfully Conjugated with AuNPs. The size of curcumin after conjugation with AuNPs was determined using dynamic light scattering. The particle size analyzer revealed that the particles formed were in the nanometer range with an average size of 53 nm (Figure 1). This confirms that the curcumin conjugated with AuNPs was successfully synthesized.

Curcumin Exhibits No Cytotoxicity toward HaCaT Cells at Concentrations below 10 μM. At 10 μM and lower, curcumin did not exhibit cytotoxic activities against human cells. At 12.5 μM, 2% cytotoxicity was recorded, while 6 and 8% were observed for 25 and 50 μM of curcumin, respectively (Figure 2). While exhibiting 11% cytotoxicity at 100 μM, curcumin exhibited 20% cytotoxicity at 200 μM. Solvent controls did not exhibit cytotoxic activities against human cells. Gold nanoparticles at both 5 and 10 μM did not exhibit cytotoxic activities against human cells. Curcumin conjugated with AuNPs at both 5 and 10 μM did not exhibit cytotoxic activities against human cells (Figure 2). Curcumin is known to be safe for humans, and it has been shown that even at doses of 8000 mg/day, curcumin did not cause any noticeable toxicities, except diarrhea and mild nausea in some cases.

![Figure 1. Size distribution of curcumin–AuNPs. The figure shows the sizes of curcumin after conjugation with AuNPs determined by dynamic light scattering.](https://dx.doi.org/10.1021/acsomega.0c01305)
Curcumin Expressed Significant Amoebicidal Activity at 50 μM against B. mandrillaris. Amoebicidal activities of curcumin against B. mandrillaris were investigated at a range of concentrations (Figure 3). The solvent control expressed 17% activity, while curcumin expressed 19, 28, 34, 37, and 55% amoebicidal activities at 12.5, 25, 50, 100, and 200 μM, respectively (Figure 3a). As compared to the solvent control, 4% ethanol (M = 17, SE = 4), curcumin exhibited significant amoebicidal activities against B. mandrillaris at 50 (M = 34, SE = 4; t(4) = 2.83, P = 0.05) and 200 μM (M = 55, SE = 6 t(4) = 5.33, P = 0.006). The activity of curcumin against B. mandrillaris is concentration dependent (Figure 3b). According to the Quest Graph IC50 calculator, the AC50 of curcumin against B. mandrillaris was 172 μM.

Curcumin Expressed a Significant Amoebicidal Activity at 50 μM against N. fowleri. Amoebicidal activities of curcumin against N. fowleri were investigated at a range of concentrations (Figure 4). At 200 μM, curcumin expressed 66% amoebicidal activity against N. fowleri (Figure 4a). As compared to the solvent control, 4% ethanol (M = 23, SE = 5),
50 (M = 41, SE = 4), 100 (M = 51, SE = 5), and 200 μM (M = 66, SE = 4) of curcumin resulted in significant (t(4) = 2.81, P = 0.05; t(4) = 4.06, P = 0.02; t(4) = 6.56, P = 0.003) amoebicidal activities against *N. fowleri*. At 6.25, 12.5, and 25 μM, curcumin expressed 22, 30, and 35% amoebicidal activities, respectively, against *N. fowleri*. The activity of curcumin against *N. fowleri* is concentration dependent (Figure 4b). The AC50 of curcumin against *N. fowleri* was 74 μM, according to the Quest Graph IC50 calculator. At 50 μM, curcumin exhibited potent amoebicidal effects against *N. fowleri* (Figure 4c).

When tested against another amoeba, *Dictyostelium discoideum*, it was shown that curcumin acts by inhibiting cell signaling, proliferation, and adhesion while increasing reactive oxygen species (ROS) by inducing the expression of glutathione S-transferase.31 The increase in ROS induced by curcumin results in both mitochondrial and nuclear DNA damage.32 Curcumin can also induce deformation due to swelling, morphological changes in cytoplasmic membrane, and cell agglutination, and provoke apoptosis-like changes.33 Moreover, the ability of curcumin to cause size changes and cell membrane damage, leading to loss of cellular integrity in
amoebae, has been reported. Curcumin has also been shown to affect gene transcription and induce apoptosis in cancer cells. Other molecular targets of curcumin include growth factors, transcription factors, protein kinases, and inflammatory cytokines. Hence, curcumin may express amoebicidal activities against *B. mandrillaris* and *N. fowleri* using one or several of these mechanisms.

Figure 4. Curcumin showed amoebicidal activities against *N. fowleri*. Briefly, *N. fowleri* was incubated with various concentrations of curcumin for 24 h, and viability was determined using trypan blue exclusion assay. The percentage amoebicidal activity was calculated by \[\frac{(\text{RPMI cell count} - \text{cell count for the sample})}{\text{RPMI cell count}} \times 100\]. A significant reduction in the number of viable *N. fowleri* cells was observed for cells treated with curcumin at 50 μM and above. The results are representative of three independent experiments performed in duplicates. The data are presented as the mean ± standard error (*: P < 0.05, **: P < 0.01, ***: P < 0.001 using Student’s t test; two-tailed distribution). (a) Bar chart showing the activity of individual concentrations and controls. (b) Curve showing the concentration-dependent activity of curcumin against *N. fowleri*. (c) Representative effects of curcumin on *N. fowleri*.

Despite its safety at high concentrations, curcumin has been shown to exhibit poor bioavailability in humans due to rapid metabolism and poor absorption. Nanoparticles can enhance the bioavailability of curcumin, a hydrophobic compound, by overcoming its low aqueous solubility. Hence, we conjugated curcumin with gold nanoparticles and tested its amoebicidal activities against *B. mandrillaris* and *N. fowleri* at 5 and 10 μM.
Figure 5. Activity of curcumin against *B. mandrillaris* was enhanced after conjugation with AuNPs. Briefly, *B. mandrillaris* was incubated with 5 and 10 μM curcumin and curcumin−AuNPs for 24 h, and viability was determined using trypan blue exclusion assay. The percentage amoebicidal activity was calculated by [(((RPMI cell count − cell count for the sample)/RPMI cell count) × 100)]. The results revealed that the activity of curcumin against *B. mandrillaris* cells increased after conjugation with AuNPs. The results are representative of three independent experiments performed in duplicates. The data are presented as the mean ± standard error (**: *P* < 0.01, ***: *P* < 0.001 using Student’s *t* test; two-tailed distribution).

Figure 6. Amoebicidal properties of curcumin against *N. fowleri* were significantly enhanced after conjugation with AuNPs. Briefly, *N. fowleri* was incubated with 5 and 10 μM curcumin and curcumin−AuNPs for 24 h, and viability was determined using trypan blue exclusion assay. Percentage amoebicidal activity was calculated by [(((RPMI cell count − cell count for sample)/RPMI cell count) × 100)]. A significant increase in the activity of curcumin against *N. fowleri* cells was observed after conjugation with AuNPs. The results are representative of three independent experiments performed in duplicates. The data are presented as the mean ± standard error (**: *P* < 0.01, ***: *P* < 0.001 using Student’s *t* test; two-tailed distribution).
Activity of Curcumin against *B. mandrillaris* was Significantly Enhanced after Conjugation with AuNPs. Amoebicidal activities of curcumin against *B. mandrillaris* after conjugation with AuNPs were investigated at 10 and 5 μM (Figure 5) since curcumin did not exhibit cytotoxicity against human cells at concentrations below 10 μM. At 10 μM, curcumin—AuNPs expressed 78% amoebicidal activities against *B. mandrillaris*. The activity of 10 μM curcumin—AuNPs (M = 78, SE = 3) was statistically significant (t(4) = 17, P = 0.00008; t(4) = 19, P = 0.00005) as compared to 10 μM AuNPs (M = 18, SE = 2), 10 μM curcumin (M = 22, SE = 0.2), and the solvent control (M = 11, SE = 1). For curcumin—AuNPs at 5 μM, 64% amoebicidal activity was observed. The amoebicidal activity of 5 μM curcumin—AuNPs (M = 64, SE = 9) as compared to 5 μM AuNPs (M = 3, SE = 5), 5 μM curcumin (M = 15, SE = 0.2), and the solvent control (M = 0, SE = 10) was statistically significant (t(4) = 5, P = 0.006; t(4) = 5, P = 0.01).

Activity of Curcumin against *N. fowleri* was Significantly Enhanced after Conjugation with AuNPs. Amoebicidal activities of curcumin conjugated with AuNPs were investigated at 5 and 10 μM. Ten-micromolar curcumin—AuNPs resulted in a 69% amoebicidal activity against *N. fowleri* (Figure 6). As compared to 10 μM AuNPs (M = 0, SE = 0), ethanol (2%) (M = 20, SE = 2), and 10 μM curcumin (M = 32, SE = 3), 10 μM curcumin—AuNPs (M = 69, SE = 1) showed significant amoebicidal activity (t(4) = 84, P = 0.0000001; t(4) = 19, P = 0.00004; t(4) = 11, P = 0.0004). At 5 μM, 53% amoebicidal activity was observed for curcumin—AuNPs. The activity of 5 μM curcumin—AuNPs (M = 53, SE = 2) was statistically significant (t(4) = 28, P = 0.000009; t(4) = 13, P = 0.0002; t(4) = 15, P = 0.0001) as compared to 5 μM AuNPs (M = 0, SE = 0), 5 μM curcumin (M = 21, SE = 2), and solvent control (M = 3, SE = 3).

Our cytotoxicity results show that curcumin did not exhibit any cytotoxic activities against human cells at 10 and 5 μM. AuNPs at both 5 and 10 μM did not exhibit any cytotoxic activities against human cells. Also, curcumin conjugated with AuNPs at both 5 and 10 μM did not exhibit cytotoxic activities against human cells. The amoebicidal activities of curcumin were enhanced from 15 and 22%, before conjugation, at 5 and 10 μM, to 64 and 78%, respectively, after conjugation, against *B. mandrillaris*. Against *N. fowleri*, the amoebicidal activities of curcumin was enhanced from 21 and 32%, before conjugation at 5 and 10 μM, to 53 and 69%, respectively, after conjugation.

The increase in activity of curcumin may be linked to the biological activity of gold nanoparticles. AuNPs have been shown to cause the formation of ROS that results in the disruption of the mitochondrial membrane potential and indicates apoptosis.\(^{37}\) AuNPs also caused a downregulation in the expression of genes involved in cell cycle and DNA repair mechanisms.\(^{36}\) Due to the drop in the mitochondrial function, the loss of mitochondrial membrane potential, the activation of caspase-3, the increase in the amount of intracellular calcium, and the increased levels of nuclear p53, AuNPs cause cell death by apoptosis.\(^{37}\) Enhanced effects of Au-conjugated drugs may also be explained by the fact that metals such as Au affect the ability of the amoebae to replicate DNA and its expression of enzymes and ribosomal subunit proteins, which are involved in the ATP production and hence respiratory chain.\(^{38}\)

In conclusion, curcumin expressed significant amoebicidal activities against both *B. mandrillaris* and *N. fowleri* at 50 μM while exhibiting limited cytotoxicity toward human cells. The activity of curcumin was significantly increased against both amoebae after conjugation with gold nanoparticles. Although the mechanism of action of curcumin against *B. mandrillaris* and *N. fowleri* is yet to be explored as well as its in vivo abilities, curcumin shows great promise as a future treatment option against those free-living amoebae.

**MATERIALS AND METHODS**

**Nanoparticle Conjugation.** Potassium gold (III) chloride and curcumin solutions were mixed in equal volume (1:1), and the solution was stirred magnetically at 200 × g for 2 h. Sodium borohydride was added to catalyze the formation of AuNPs from the potassium gold (III) chloride. As previously described, curcumin—AuNPs complex formation was indicated by color change of solution from colorless to pale pink.\(^{39}\) Dynamic light scattering (Litesizer 500, Anton Paar) was performed at 25°C and a constant angle of 90° to analyze the size distribution profile of curcumin—AuNPs in a suspension at 25°C and a constant angle of 90°, as previously described.\(^{40}\)

**Henrietta Lacks Cervical Cancer Cells.** Henrietta Lacks (HeLa) cervical cancer cells were used as a food source for amoeba cells.\(^{42}\) HeLa cells were acquired from American Type Culture Collection (ATCC CCL-2, Singapore). Cells were cultured in a Roswell Park Memorial Institute (RPMI) 1640 medium (Serana, Germany), supplemented with 10% fetal bovine serum (FBS) (Sigma, United States), 1% antibiotics (penicillin–streptomycin) (Nacalai Tesque, Japan), 1% minimum essential medium amino acids (Nacalai Tesque, Japan), and 1% l-glutamine (Nacalai Tesque, Japan) at 5% CO\(_2\), 95% humidity, and 37°C as previously described.\(^{42}\)

**Human Keratinized Skin Cells.** Human keratinized skin cells (HaCaT) (CLS:300493) were obtained from CLS Cell Lines. HaCaT cells were grown in RPMI-1640 (Serana, Germany) complemented with 10% FBS (Sigma, United States), 1% antibiotics (penicillin–streptomycin) (Nacalai Tesque, Japan), 1% minimum essential medium amino acids (Nacalai Tesque, Japan), and 1% l-glutamine (Nacalai Tesque, Japan) at 37°C and 5% CO\(_2\).

**N. fowleri Culture.** *N. fowleri* was cultured as previously described.\(^{41}\) *N. fowleri* cells (HB1 strain; ATCC 30174) were acquired from ATCC and cultured in an RPMI-1640 medium (Serana, Germany) supplemented with 1% antibiotics (penicillin–streptomycin) (Nacalai Tesque, Japan) with a monolayer of HeLa cells used as a food source at 5% CO\(_2\) and 37°C.

**B. mandrillaris Culture.** *B. mandrillaris* cells (ATCC S0209) were acquired from ATCC and cultured in an RPMI-1640 (Serana, Germany) medium supplemented with 1% antibiotics (penicillin–streptomycin) (Nacalai Tesque, Japan) with a monolayer of HeLa cells used as a food source at 5% CO\(_2\) and 37°C, as previously described.\(^{43}\)

**Cytotoxicity Assay.** Cytotoxicity of curcumin against human cells was assessed as previously described.\(^{42,44}\) Various concentrations of curcumin were incubated in RPMI-1640 (Serana, Germany) with HaCaT cells for 24 h at 5% CO\(_2\) and 37°C. Cell-free supernatant media was collected, and the presence of LDH enzyme was assessed using a cytotoxicity detection kit (Roche Applied Science). Percentage cytotoxic effects were determined as follows: [(absorbance of media from cells treated with sample − absorbance of media from untreated cells)/(absorbance of media from cells with total LDH release − absorbance of media from untreated cells)] × 100% = percentage cytotoxic activity.
Amoebicidal Assay. The ability of the compounds to kill the amoebae was determined through amoebicidal assays, as previously described. In 24-well plates in RPMI-1640 (Serana, Germany), $5 \times 10^5$ amoeba cells were incubated with curcumin, curcumin–AuNPs complexes, and solvents at 37 °C for 24 h. Amoeba incubated with RPMI-1640 served as a negative control. For the positive control, miltefosine was used. The percentage of viable amoebae was determined by counting unstained (live) amoeba cells using a hemocytometer, following the addition of 0.1% trypan blue. Statistical significance was calculated using Student’s t test with two-tailed distribution to compare the mean of experimental results.

Statistical Analysis. The data are illustrative of the mean ± standard error of several independent experiments accomplished in duplicates. Statistical significance for differences was evaluated using a two-sample t test with two-tailed distribution, contrasting the mean of two different experiments repeated using similar conditions. P values were determined for analysis.

AUTHOR INFORMATION

Corresponding Author
Naveed Ahmed Khan — Department of Biology, Chemistry and Environmental Sciences, College of Arts and Sciences, American University of Sharjah, Sharjah 26666, United Arab Emirates; orcid.org/0000-0001-7667-8553; Phone: +971-6515-4752; Email: naveed5438@gmail.com

Authors
Mohammad Ridwane Mungroo — Department of Biological Sciences, Sunway University, Bandar Sunway 47500, Malaysia
Ayaz Anwar — Department of Biological Sciences, Sunway University, Bandar Sunway 47500, Malaysia
Ruqaiyyah Siddiqui — Department of Biology, Chemistry and Environmental Sciences, College of Arts and Sciences, American University of Sharjah, Sharjah 26666, United Arab Emirates

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c01305

Author Contributions
A.A. and R.S. established the idea and supervised the study. M.R.M. synthesized molecules and characterized nanoparticles under the supervision of A.A. M.R.M. conducted all the bioassays and prepared the first draft of the manuscript under the supervision of R.S. and N.A.K. N.A.K. and R.S. corrected and finalized the manuscript for submission. The manuscript was submitted with the endorsement of all authors.

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS
The work in this paper was supported, in part, by the Open Access Program from the American University of Sharjah. This paper represents the opinions of the author(s) and does not mean to represent the position or opinions of the American University of Sharjah.

REFERENCES
(1) Martinez, A. J. Free-living amoebas; natural history, prevention, diagnosis, pathology and treatment of disease. CRC Press: Ohio, 1985; pp 1–166.
(2) Schuster, F. L.; Visvesvara, G. S. Free-living amoebae as opportunistic and non-opportunistic pathogens of humans and animals. Int. J. Parasitol. 2004, 34, 1001–1027.
(3) Visvesvara, G. S. Infections with free-living amebae. In Handbook of clinical neurology, Elsevier: Amsterdam, 2013, 114, pp. 153–168, DOI: 10.1016/B978-0-444-53490-3.00010-8.
(4) Anwar, A.; Siddiqui, R.; Shah, M. R.; Khan, N. A. Gold nanoparticle-conjugated cinnamic acid exhibits anticanthamoebic and antibacterial properties. Antimicrob. Agents Chemother. 2018, 62, e00630-18.
(5) De Jonckheere, J. F. Origin and evolution of the worldwide distributed pathogenic amoebodagellate Naegleria fowleri. Infect., Genet. Evol. 2011, 11, 1520–1528.
(6) Mungroo, M. R.; Khan, N. A.; Siddiqui, R. Naegleria fowleri: diagnosis, treatment options and pathogenesis. Expert Opin. Orphan Drugs 2019, 7, 67–80.
(7) Heggie, T. W. Swimming with death: Naegleria fowleri infections in recreational waters. Travel Med. Infect. Dis. 2010, 8, 201–206.
(8) Mungroo, M. R.; Anwar, A.; Khan, N. A.; Siddiqui, R. Brain- eating Amoebae Infection: Challenges and Opportunities in Chemo- therapy. Mini-Rev. Med. Chem. 2019, 19, 980–987.
(9) Sau, K.; Mambula, S. S.; Latz, E.; Henneke, P.; Golenbock, D. T.; Levitz, S. M. The antifungal drug amphotericin B promotes inflammatory cytokine release by a Toll-like receptor-and CD14- dependent mechanism. J. Biol. Chem. 2003, 278, 37561–37568.
(10) Kim, J. H.; Jung, S. Y.; Lee, Y. J.; Song, K. J.; Kwon, D.; Kim, K.; Park, S.; Im, K. I.; Shin, H. J. Effect of therapeutic chemical agents in vitro and on experimental meningoencephalitis due to Naegleria fowleri. Antimicrob. Agents Chemother. 2008, 52, 4010–4016.
(11) Visvesvara, G. S.; Moura, H.; Schuster, F. L. Pathogenic and opportunistic free-living amoebae: Acanthamoeba spp., Balamathia mandrillaris, Naegleria fowleri, and Sappinia diploidea. FEMS Immunol. Med. Microbiol. 2007, 50, 1–26.
(12) Kalsoom, H.; Baig, A. M.; Khan, N. A.; Siddiqui, R. Laboratory testing of clinically approved drugs against Balamathia mandrillaris. World J. Microbiol. Biotechnol. 2014, 30, 2337–2342.
(13) Laurie, M. T.; White, C. V.; Retallack, H.; Wu, W.; Moser, M. S.; Sakanari, J. A.; Ang, K.; Wilson, C.; Arkin, M. R.; DeRisi, J. L. Functional Assessment of 2,177 U.S. and International Drugs Identifies the Quinoline Nitroxoline as a Potent Amoebicidal Agent against the Pathogen Balamathia mandrillaris. mBio. 2018, 9, e02051-18.
(14) Siddiqui, R.; Khan, N. A. Balamathia amoebic encephalitis: an emerging disease with fatal consequences. Microb. Pathog. 2008, 44, 89–97.
(15) Aggarwal, B. B.; Kumar, A.; Bharti, A. C. Anticancer potential of curcumin: preclinical and clinical studies. Anticancer Res. 2003, 23, 363–398.
(16) Anand, P.; Kunnunakkara, A. B.; Newman, R. A.; Aggarwal, B. B. Bioavailability of curcumin: problems and promises. Mol. Pharmacol. 2007, 4, 807–818.
(17) Sharma, O. P. Antioxidant activity of curcumin and related compounds. Biochem. Pharmacol. 1976, 25, 1811.
(18) Ruby, A. J.; Kuttan, G.; Babu, K. D.; Rajasekharan, K. N.; Kuttan, R. Anti-tumour and antioxidant activity of natural curcuminoids. Cancer Lett. 1995, 94, 79–83.
(19) Srim, R. C.; Dhawan, B. N. Pharmacology of diterolufy methane (curcumin), a non-steroidal anti-inflammatory agent. J. Pharm. Pharmacol. 1973, 25, 447–452.
(20) Jordan, W. C.; Drew, C. R. Curcumin—a natural herb with anti-HIV activity. J. Natl. Med. Assoc. 1996, 88, 333.
(21) Mahady, G. B.; Pendland, S. L.; Yun, G.; Lu, Z. Z. Turmeric (Curcuma longa) and curcumin inhibit the growth of Helicobacter pylori, a group 1 carcinogen. Anticancer Res. 2002, 22, 4179–4181.
(22) Kim, M. K.; Choi, G. J.; Lee, H. S. Fungicidal property of Curcuma longa L. rhizome-derived curcumin against phytopathogenic fungi in a greenhouse. J. Agric. Food Chem. 2003, 51, 1578–1581.
(23) Reddy, R. C.; Vatsala, P. G.; Keshamouni, V. G.; Padmanaban, G.; Rangarajan, P. N. Curcumin for malaria therapy. Biochem. Biophys. Res. Commun. 2005, 326, 472–474.
(24) Cheraghapur, K.; Marzban, A.; Ezatpour, B.; Khanizadeh, S.; Koshki, J. Antiparasitic properties of curcumin: A review. AIMS Agric. Food 2018, 4, 561.
(25) Kuttan, R.; Bhanumathy, P.; Nirmala, K.; George, M. C. Potential anticancer activity of turmeric (Curcuma longa). Cancer Lett. 1985, 29, 197–202.
(26) Aqeel, Y.; Iqbal, J.; Siddiqui, R.; Gilani, A. H.; Khan, N. A. Anti-Acanthamoebic properties of resveratrol and demethoxycurcumin. Exp. Parasitol. 2012, 132, 519–523.
(27) El-Sayed, N. M.; Ismail, K. A.; Ahmed, S. A. E. G.; Hetta, M. H. In vitro amoeboidal activity of ethanol extracts of Arachis hypogaea L., Curcuma longa L and Pancreatum marinum L. on Acanthamoeba castellani cysts. Parasitol. Res. 2012, 110, 1985–1992.
(28) Cole, G. M.; Teter, B.; Frautschy, S. A. Neuroprotective effects of curcumin. In The molecular targets and therapeutic uses of curcumin in health and disease; Springer: New York, 2007, pp. 197–212.
(29) Maheshwari, R. K.; Singh, A. K.; Gaddipati, J.; Srimal, R. C. Multiple biological activities of curcumin: a short review. Life Sci. 2006, 78, 2081–2087.
(30) Hsu, C. H.; Cheng, A. L. Clinical studies with curcumin. In The molecular targets and therapeutic uses of curcumin in health and disease. Springer: New York, 2007, pp. 471–480.
(31) Garige, M.; Walters, E. Curcumin inhibits development and cell adhesion in Dictyostelium discoideum: Implications for YakA signaling and GST enzyme function. Biochem. Biophys. Res. Commun. 2015, 407, 275–281.
(32) 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, 488–494.
(33) Pérez-Arriaga, L.; Mendoza-Magaña, M. L.; Cortés-Zárate, R.; Corona-Rivera, A.; Bobadilla-Morales, L.; Troyo-Sanromán, R.; Ramírez-Herrera, M. A. Cytotoxic effect of curcumin on Giardia lamblia trophozoites. Acta Trop. 2006, 98, 152–161.
(34) Rangel-Castañeda, I. A.; Hernández-Hernández, J. M.; Pérez-Rangel, A.; González-Pozos, S.; Carranza-Rosales, P.; Charles-Niño, C. L.; Tapia-Pastrana, G.; Ramírez-Herrera, M. A.; Castillo-Romero, A. Amebicidal activity of curcumin on Entamoeba histolytica trophozoites. J. Pharm. Pharmacol. 2018, 70, 426–433.
(35) Sharma, R. A.; Gescher, A. J.; Steward, W. P. Curcumin: the story so far. Eur. J. Cancer 2005, 41, 1955–1968.
(36) Zhou, H.; S. Beever, C.; Huang, S. The targets of curcumin. Curr. Drug Targets 2011, 12, 332–347.
(37) Schaeublin, N. M.; Braydich-Stolle, L. K.; Schrand, A. M.; Miller, J. M.; Hutchison, J.; Schlager, J. J.; Hussain, S. M. Surface charge of gold nanoparticles mediates mechanism of toxicity. Nanoscale 2011, 3, 410–420.
(38) Aqeel, Y.; Siddiqui, R.; Anwar, A.; Shah, M. R.; Khan, N. A. Gold nanoparticle conjugation enhances the antiacanthamoebic effects of chlorhexidine. Antimicrob. Agents Chemother. 2016, 60, 1283–1288.
(39) Anwar, A.; Mungroo, M. R.; Anwar, A.; Sullivan, W. J., Jr.; Khan, N. A.; Siddiqui, R. Repositioning of guanabenz in conjugation with gold and silver nanoparticles against pathogenic amoebae Acanthamoeba castellani and Naegleria fowleri. ACS Infect. Dis. 2019, 5, 2039–2046.
(40) Anwar, M. F.; Yadav, D.; Kapoor, S.; Chander, J.; Samim, M. Comparison of antibacterial activity of Ag nanoparticles synthesized from leaf extract of Parthenium hystrophorus L in aqueous media and gentamicin sulphate: in-vitro. Drug Dev. Ind. Pharm. 2015, 41, 43–50.
(41) Rajendran, K.; Anwar, A.; Khan, N. A.; Siddiqui, R. Brain-eating amoebae: silver nanoparticle conjugation enhanced efficacy of anti-amoebic drugs against Naegleria fowleri. ACS Chem. Neurosci. 2017, 8, 2626–2630.
(42) Siddiqui, R.; Jeyamogan, S.; Ali, S. M.; Abbas, F.; Sagathevan, K. A.; Khan, N. A. Crocodiles and alligators: Antiamoebic and antitumor compounds of crocodiles. Exp. Parasitol. 2017, 183, 194–200.
(43) Siddiqui, R.; Matin, A.; Warhurst, D.; Stins, M.; Khan, N. A. Effect of antimicrobial compounds on Balamuthia mandrillaris encystment and human brain microvascular endothelial cell cytopathogenicity. Antimicrob. Agents Chemother. 2007, 51, 4471–4473.
(44) Khan, N. A. Pathogenicity, Morphology, and Differentiation of Acanthamoeba. Curr. Microbiol. 2001, 43, 391–395.