Original Article

In vitro activity of *Curcuma longa* Extract Against the Promastigote Stage of Cutaneous *Leishmania* Parasite

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Abstract

Background: Plants are an excellent source of herbal medicines, and their natural products have antimicrobial potential for the management of numerous diseases, such as leishmaniasis. **Objectives:** This study investigated the antileishmanial activity of *Curcuma longa* plant extracts (alcoholic and aqueous extracts) against the promastigote form of cutaneous *Leishmania* parasite during 9 days of growth, as well as to investigate the effects of metronidazole and cotrimoxazole drugs on promastigote stages in different concentrations. **Materials and Methods:** To investigate cutaneous *Leishmaniasis* (CL) parasites *in vitro* using cultures of parasites. Using 0.5 g/100 mL of *C. longa* plant extracts (alcoholic and aqueous) as minimum inhibitory concentration poses significant activity on promastigate stages of the CL parasite. Two drugs were used, metronidazole and cotrimoxazole, in concentrations of 7,500 and 10,000 mg/mL. **Results:** All three extracts of the *C. longa* plant exhibited antileishmanial activity on promastigate stages of the parasite during 9 days of growth. *Curcuma longa* plant alcoholic extracts in different concentrations (0.5, 1, 1.5, 2) g/100 mL reduced the growth of promastigate stages of the CL parasite during 9 days. Also at the same concentrations of *C. longa* aqueous extract (hot water), the growth of promastigate stages was considerably decreased during 9 days. *Curcuma longa* alcoholic plant extract was found to be most active against promastigate stages of CL compared to aqueous extracts. The two drugs used, metronidazole and cotrimoxazole, at concentrations of 7,500 mg/mL and 10,000 mg/mL), completely killed the promastigate stages of CL by the sixth and fifth day of treatment. **Conclusions:** Curmeric plant extracts showed inhibitory efficacy toward promastigate stages of cutaneous *Leishmania* parasite, and therefore used as an alternative treatment against parasites associated with CL.

Keywords: Antileishmanial activity, *Curcuma longa*, *Leishmania tropica*, plant extract

Introduction

Cutaneous leishmaniasis (CL) is an endemic disease in Iraq. It is mostly seen in Baghdad.[1] It is known as the " little sister" in regions where the disease is so common that it is part of the family.[2] CL is caused by two species of *Leishmania*, *L. major* causing a rural wet type with early ulceration, and other is *L.tropica* causing an urban dry type that runs a chronic course with late ulceration.[3] The disease starts with a papule that enlarges and becomes an ulcer. The lesions may be single or multiple and can be diffuse lesions. They may heal spontaneously within weeks to months or last for a year or more.[4,5]

World Health Organization ranks CL as one of the 10 most important infectious diseases worldwide.[6,7] The treatment of CL is done through the administration of pentavalent antimonials such as Pentosan® and Glucantime®, or, in more severe cases, use of Amphotericin B. However, these drugs present several side effects, such as nausea, vomiting, and cardiac disorders, among others; in addition, presenting restrictions on their use by patients with heart problems or pregnant women.[8,9]

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Recently, phytotherapy has received considerable attention as an alternative to chemotherapy in the treatment of parasitic diseases.[10] 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 a food spice and colorant. Curcumin is a polyphenolic, nontoxic, and pharmacologically active substance that has antioxidant, anti-inflammatory, and antiparasitic activities. For centuries, it has been used as a therapeutic agent against different diseases.[11] Antiprotozoal activities of curcumin have also been identified both *in vitro* and *in vivo* for *Plasmodium*,[12] *Leishmania*,[13-15] *Trypanosoma*,[16] and *Giardia lamblia*.[17] 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 and it has been accepted as a safe compound without toxic effects by the food and drug administration.[18] In the present study, an attempt was made to evaluate curcumin's leishmanicidal activity on the promastigotes stages of CL.

Materials and Methods

Parasite culture

To obtain the parasite culture *Leishmania* parasites from the samples, 10.43 g/1000 mL of RPMI 1640 medium was dissolved in sterile distilled water and distributed among 20 vials of culture tubes, each having 5 mL of the dissolved media supplemented with 10% fetal calf serum.[19] Antibiotics consisting of Penicillin G and Kanamycin were added to the culture medium to avoid bacterial and fungal contaminations. The skin scrapings were directly mixed from the lesions of infected patients into each culture tube containing 0.9% normal saline, which was placed in an ice jar and transported to the Zoology Department laboratory, Kohat University of Science and Technology (KUST). These samples were kept in an incubator (Memmert type INB 500, Germany) at 26 ± 10°C to avoid contamination. After 4–6 days of incubation, culture was observed using Giemsa-stained smears for the growth of *Leishmania* promastigotes under an Olympus microscope at 10X, 40X, and 100X.

Viability test for cutaneous *Leishmania* parasite

It was done using Erythrosine-B stain (0.4%) as follows:

Equal volumes (100 microliters) of both the parasitic suspension and the Erythrosine-B stain (0.4%), which was diluted in phosphate buffer were mixed. The mixture was kept cold and stored at 4°C in ice for 5 min. A drop of the suspension was tested and at least 100 cells were counted to estimate the viability percentage of the parasite cells with the use of a hemocytometer. The stained parasitic cells indicated dead cells, whereas the unstained ones indicated viability. Each sample was tested in five repeats for each concentration.

Effect of plant extracts of *C. longa* plant on promastigote of CL parasite

After culturing and propagating the *Leishmania* parasite, the cells were counted and maintained using a hemocytometer. It was found that there were about 500,000 flagellated parasites per milliliter in the cultured medium. The estimation of parasitic viability was assessed at the beginning and at the end of the experiment. Approximately 0.5 milliliters of the parasitic suspension in the Novy-MCNeal-Nicolle culture medium was then transferred to the culture medium of RPMI 1640.

For accomplishing the experiment, the glass bottles containing the culture media and a parasite suspension were treated with different concentrations of plant extracts: 0.5, 1, 1.5, and 2 gm/100 mL with five repeats for each concentration. Five untreated cultured bottles were kept as a control group.

The mean parasite viability percentages were counted at the beginning and at the end of the experiment by using the index of the Erythrosine-B stain.

After preparing the concentrations and shaking them well with the parasite suspension, each concentration was transferred separately for incubation at 26º C in a refrigerator. After this, the parasite viability and numbers were counted. Also, this was done to understand and assess the effect of plant extracts on the viability of parasites during a 9-day post-treatment period. The experiment was carried out by using:

A. Hot water plant extract of *C. longa* in the following concentrations: 0.5, 1, 1.5, 2 g/100 mL.
B. Cold water plant extract of *C. longa* in the following concentrations: 0.5, 1, 1.5, 2 g/100 mL.
C. Alcoholic plant extract of *C. longa* in the following concentrations: 0.5, 1, 1.5, 2 g/100 mL.

Treatments by chemical drugs—metronidazole—and cotrimoxazole

The experiment was carried out by using the following chemical drugs:

A. Metronidazole drug: 200 mg was suspended in 10 mL of sterile saline solution with a concentration of 0.15 M NaCl.
B. Cotrimoxazole drug: 80–400 mg was suspended in 10 mL of sterile saline solution with a concentration of 0.15 M NaCl.

The experiment was carried out by using the same method as that used for preceding plant extracts, but with concentrations of 7500 mg/mL and 1000 mg/mL for the metronidazole drug. Whereas, the concentration used

for cotrimoxazole was used at a steady concentration of 1000 mg/mL.

Statistical analysis

Statistical analysis was done using Statistical Package for Social Science version 25 (SPSS, IBM Company, Chicago, IL 60606, USA) and Microsoft Office Excel 2010. Numeric data were presented as mean, standard deviation, median, and interquartile range (IQR), while nominal data were expressed as numbers and percentages. Chi-square test was used to compare one numeric and one nominal data group. Mann–Whitney *U* test was used to compare median values between two non-parametric groups. Correlation coefficients were estimated by Spearman correlation and Pearson correlation.

Results

The 0.5, 1, 1.5, and 2 g/100 mL concentrations of *C. longa* alcoholic and aqueous extracts were used to determine the impact on the development of CL.

Inhibitory effect of *C. longa* extracts on the vitality of the promastigote of CL

Alcoholic extract

The significant effect of different concentrations of alcoholic extract of turmeric on the average number and vitality of promastigote stages was observed, with significant differences at the probability level of $P < 0.05$ as shown in Table 1.

The concentration of 2 g/100 mL showed the highest significant effect on the seventh day of treatment, causing 100% mortality of live parasites, while the vitality of promastigote stages was 55% after 2 days of treatment when compared to the control group (120 g/ 100 mL). The concentration of 1.5 g/100 mL showed after 7 days of treatment, causing 100% mortality of live parasites. The vitality of promastigote stages was 60% after 2 days of treatment. With the concentration of 1 g/100 mL, a significant effect was observed after 8 days of treatment, resulting in vitality of promastigote stages to 2%, while it was 65% after 2 days of treatment. When using the concentration of 0.5 g/100 mL, the highest effect was recorded after 8 days of treatment, with the viability of promastigote stages was 6%, while it was 75% after 2 days of treatment when compared with the control (120 µg/ mL).

Aqueous extracts

Hot water

Table 2 shows the significant effect of different concentrations of the hot water extract of turmeric on the number and vitality of the promastigote stages, and the presence of significant differences between treatments at a probability level of $P < 0.05$.

The concentration of 2 g/100 mL showed the highest significant effect on the seventh day of treatment, causing

Table 1: The effect of different concentrations of alcoholic extracts of *Curcuma longa* plant in the viability range of promastigate stages during 9 days of growth

Time in days Con. g/100mL	Viability mean of promastigote stages									M± SD
	1	2	3	4	5	6	7	8	9	
Control	120	120	120	120	120	120	120	120	120	120 ± 0
0.5	95	75	60	45	35	25	15	6	0	35.7 ± 34.8
1	85	65	55	40	30	20	10	2	0	30.8 ± 31.7
1.5	80	60	45	35	25	12	4	0	0	25.1 ± 28
2	75	55	40	30	20	6	0	0	0	21.2 ± 29.8

Reading represents the mean of five repeats ± SD, LSD = 3.3

Table 2: The effect of different concentrations of hot water extracts of *Curcuma longa* plant in the viability range of promastigote stages during 9 days of growth

Time in days Con. g/100mL	Viability mean of promastigote stages									M± SD
	1	2	3	4	5	6	7	8	9	
Control	120	120	120	120	120	120	120	120	120	120 ± 0
0.5	105	95	80	70	55	40	25	12	2	53.8 ± 36.6
1	95	85	65	50	35	20	8	2	0	40 ± 35.8
1.5	90	75	60	45	30	15	6	0	0	35.7 ± 30.7
2	85	70	55	40	25	10	0	0	0	34.4 ± 29.3

Reading represents the mean of five repeats ± SD, LSD = 5.4

100% mortality parasite mortality, while on the second day of treatment, the percentage of viability of promastigote stages of CL reduced to 70% equilibrium compared with the control (120 g/100 mL). With the concentration of 1.5 g/ 100 mL, a higher effect was observed after 7 days, with the vitality of promastigote stages of CL at 6%, compared to 75% after 2 days of treatment. The concentration of 1 g/100 mL showed a significant effect on the viability percentage of promastigote stages after 8 days, causing 2% viability, while on the second day of treatment, the percentage of viability was 85%. The concentration of 0.5 g/100 mL showed a significant effect on the viability percentage of promastigote stages on the ninth day of treatment, which was 2%, compared to 95% after 2 days of treatment when compared with the control (120 µg/mL).

Regarding the overall mean of the concentrations, the lowest concentration in this study was 0.5 g/mL showed significant differences at a probability level of $P < 0.05$ for days (8, 7, 6, 5, 4, 3, 2, 1).

Cold water

Table 3 shows the significant effect of different concentrations of the cold water extract of turmeric on the number and viability of promastigote stages and the presence of significant differences between treatments at a probability level of $P < 0.05$.

The concentration of 2 g/100 mL showed the highest significant effect on the eighth day of treatment, causing 100% mortality of all parasites, while on the second day of treatment, the percentage of viability of promastigote stages of CL reduced to 70% compared to the control (120 g/100 mL). After 8 days of treatment using the concentration 1.5 g/100 mL, it appeared a higher record, as the viability of promastigote stages of CL was 6%, whereas it was 85% after 2 days of treatment. The concentration of 1 g/100 mL showed a significant effect on the percentage of viability of promastigote stages after 8 days, with viability at 12%, compared to 90% after the second day of treatment. The concentration of 0.5 g/100 mL showed a significant effect on the percentage of viability of promastigote stages after 9 days of treatment was 2%, compared to 100% after 2 days of treatment when compared with the control (120 µg/mL).

Regarding the overall average of the concentrations used in this study, the concentration of 0.5 g/mL differed from the other concentrations at the probability level $P < 0.05$ in which the viability decreased to 62.6%.

Sensitivity of promastigote stages of CL to antibiotics (metronidazole and cotrimoxazole)

The results in Table 4 showed the significant effect of each of the concentrations 7,500 and 10,000 µg/mL of metronidazole and cotrimoxazole (1000 µg/mL), where clear differences were observed.

The concentration of 7500 µg/mL showed complete inhibition of promastigote stages of CL, and they showed a 100% kill rate on the sixth day of treatment, and the viability of promastigote stages decreased significantly to 10% on the fourth day of treatment when compared to the control (120 µg/mL).

While the concentration of 10,000 µg/mL showed a complete inhibitory effect for the promastigote stages,

Table 3: The effect of different concentrations of cold water extracts of *C. longa* in the viability range of promastigote stages during 9 days of growth

Time in days Con. g/100ml	Viability mean of promastigate stages									M± SD
	1	2	3	4	5	6	7	8	9	
Control	120	120	120	120	120	120	120	120	120	120 ± 0
0.5	110	100	95	80	65	50	35	20	2	62.6 ± 36.6
1	100	90	80	65	50	40	25	12	0	51.6 ± 34.8
1.5	95	85	70	55	45	30	15	6	0	44.6 ± 34.4
2	90	70	55	35	20	12	2	0	0	31.6 ± 33.2

Reading represents the mean of five repeats ±SD, LSD = 10.8

Table 4: The effect of different concentrations of chemical drugs—metronidazole and cotrimoxazole in the viability range of promastigate stages during 6 days of growth

Time in days Con. mg/mL	Viability mean of promastigote stages						M± SD
	1	2	3	4	5	6	
Control	120	120	120	120	120	120	120 ± 0
10000	80	25	10	2	0	0	19.5 ± 31.1
7500	90	70	30	10	2	0	33.7 ± 38

Reading represents the mean of five repeats ±SD, LSD = 11.2

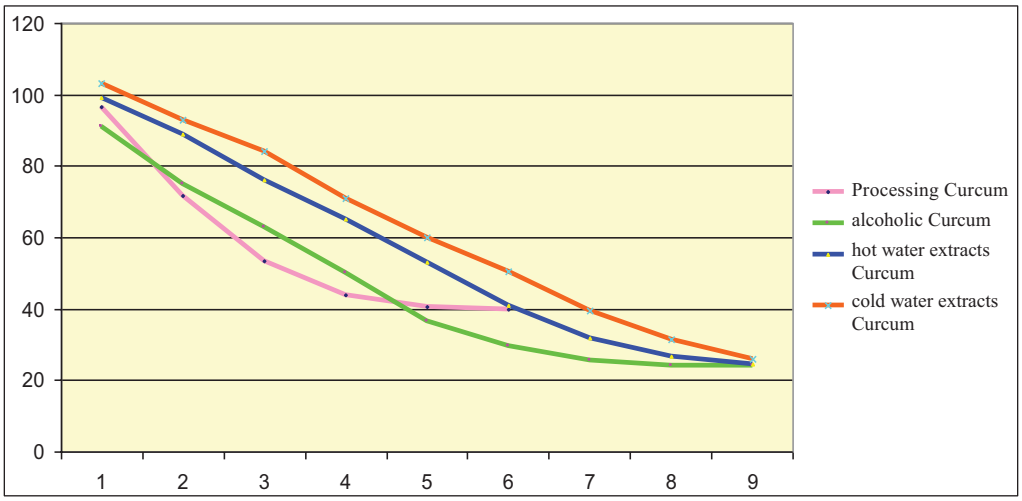

Figure 1: Relationship between alcoholic extract, hot and cold water extract with treatment with metronidazole, cotrimoxazole, and turmeric

showing a 100% kill rate on the fifth day of treatment for the mentioned concentrations; the viability of promastigote stages decreased significantly to 10% on the third day of treatment when compared with the control (120 µg/mL).

Figure 1 shows a summary comparison between the effectiveness of alcoholic and aqueous plant extracts (hot and cold) of the turmeric plant, and therapeutic consisting of metronidazole at a concentration of 7500 and 1000 µg/mL additives for each concentration of 1000 µg/mL of cotrimoxazole.

The comparison of the mentioned plant extracts showed that the effective concentration was 2 g/100 mL, which completely killed the promastigote stages of CL on the seventh day of treatment with the alcoholic extract of turmeric, on the eighth day of treatment with cold water extract, and on the seventh day of treatment with hot water extract of turmeric. In the case of chemotherapy, the concentrations of 7,500 and 10,000 mg/mL (metronidazole and cotrimoxazole) completely killed the promastigote stages of CL on the sixth and fifth day of treatment.

Discussion

Leishmaniasis is an important parasitic disease all around the world. Due to the resistance of *Leishmania* strain to pentavalent antimonials, there is a great need for the development of a new, effective, and safe drug for its treatment. Most drugs currently used for *Leishmaniasis* have several limitations, such as high toxicity, challenging treatment schedules, and development of resistance.[20] Therefore, there is an urgent need for new, safe, more effective, and economically feasible drugs for the treatment of *Leishmaniasis*. For reducing the resistance of *Leishmanias* strains against drugs, alternative strategies, such as the use of herbal plants are considered.[21,22] Herbal products have been used for the treatment of multiple human diseases for thousands of years such as curcumin.

Curcumin is a natural pharmacologically active agent and has several anti-inflammatory mechanisms that may be detrimental to effective host control of intracellular microbial infections.[23,24] In our study, we have provided clear evidence that curcumin may constitute a potent antileishmanial agent against promastigote stages of CL *in vitro*.

The 0.5 g/100 mL concentration of *C. longa* alcoholic extracts resulted in the eradication of the parasites within 9 days of treatment; in addition, 1 g/100 mL concentration completed the inhibition within 9 days of treatment. The 1.5 g/100 mL concentration completed the inhibition within 8 days of treatment, and the 2 g/100 mL concentration completed the inhibition within 7 days of treatment. After 2 days, the concentrations of 0.5, 1, 1.5, and 2 g/100 mL of *C. longa* plant alcoholic extract reduced the growth rate to 75%, 65%, 60%, and 55%, respectively. After 9 days, all concentrations resulted in complete inhibition in the culture media compared to the control group.

The 2 g/100 mL concentration of *C. longa* hot water extracts resulted in the eradication of the parasites within 7 days of treatment, while the 1.5 g/100 mL concentration completed the inhibition within 8 days of treatment. The 1 g/100 mL concentration completed the inhibition within 9 days of treatment, while the 0.5 g/100 mL concentration did not show any inhibition of the parasites. After 2 days, the concentrations of 0.5, 1, 1.5, and 2 g/100 mL of *C. longa* plant hot water extracts reduced the growth rate to 95%, 85%, 75%, and 70%, respectively. After 9 days, all concentrations resulted in complete inhibition in the culture media except for the 0.5 g/100 mL concentration when compared with the control group.

The 2 g/100 mL concentration of *C. longa* plant cold water extract resulted in the eradication of the parasites

within 8 days of treatment, while the 1.5 g/100 mL and 1 g/100 mL concentrations completed the inhibition within 9 days of treatment, while the 0.5 g/100 mL did not show any inhibition of the parasites. After 2 days, the concentrations of *C. longa* plant cold water extract (0.5, 1, 1.5, and 2 g/100 mL) reduced the growth rate to 100%, 90%, 85%, and 70%, respectively. After 9 days, all concentrations resulted in complete inhibition in the culture media, except 0.5 g/100 mL concentration when compared with the control group.

In this study, the alcoholic extract of *C. longa* plant showed the highest effect against the cutaneous *Leishmania* parasite, as the vitality of promastigote stages was decreased when compared with control; our results were similar to the one observed by Rasmussen and his group,[25] who reported the efficacy of an ethanolic extract from *C. longa* against *L. major*, which was able to inhibit the growth of these parasites. In another study, ethanolic and methanolic extracts of *Perovskia abrotanoides* root were found to have antileishmanial activity and kill *L. major* promastigotes,[26] while Khalid and his group[27] indicated that methanolic extract of leaves and fruits of *Azadirachta indica* had an antileishmanial activity on promastigote stages of *L. major*.

Also, the results of this study demonstrate the clear inhibitory impact of the aqueous (hot) extract of the *C. longa* plant on the growth of *Leishmania.* The findings clearly show that it can kill these parasites and can therefore be employed as a possible treatment for the infection.

In the treatment at high concentration levels, the infection in the culture media was significantly reduced. In addition, the growth rate was decreased with increasing concentration levels during different periods of treatment, a result similar to the one observed by Iqbal *et al*.[28]

The inhibitory activity of curcumin against promastigote (extracellular) forms of *Leishmania cutaneous* parasite is due to the plant containing many compounds, which have the capacity to directly affect the centers of enzyme production and other vital components, thus causing the annihilation of the cells and, consequently, elimination, as well as, the plant contains a number of benzylisoquinolines, the ß-carboline alkaloids, the iridoid, steroidal glycosides, quinines,[29] and tannins, which are phenolic compounds and significantly affect the acetylcholinesterase enzyme. This enzyme controls all physiological events and the passage of ions to and from the cell through the membrane of the cell, thus controlling the mobility of the parasite, inhibition of this enzyme will lead to the death of the parasite.[30]

The results showed that the effective concentration of treatment with the alcoholic and, hot and cold water extract of turmeric is 2 g/100 mL; after 6 days, a statistically significant decrease in the number and vitality of promastigote stages of CL was observed, while in the control the growth continued. The examinations showed that using higher concentrations of alcoholic and hot water extract of *C. longa* plant caused more decrease in the number and vitality of promastigote stages on the seventh day due to the presence of hydroxyl and phenol groups in the turmeric molecule, which are essential for the inhibition of parasites.[31-33]

The results of this study showed that the alcoholic extract of *C. longa* plant has higher activity than the aqueous (hot and cold) extract in inhibition of the growth of *Leishmania* promastigote (extracellular) forms, which is related to the nature of the active compounds (volatile oils) and also the solvents used in the extraction.[34] Oils are nonpolar compounds that do not easily dissolve in water but they dissolve in nonpolar organic solvents such as ethanol.[35]

The inhibitory effect of the alcoholic extract over the aqueous extract may explain the ability of ethanol to dissolve a number of active substances such as terpenes and collect them in the crude extract in sufficient quantities to cause the inhibitory action.[36] It may be explained that the active ingredients of the turmeric plant may be dissolved in alcohol and not in the water, so the alcoholic extract will be rich in the main active compounds of the turmeric plant that have anti-leishmanial activity against *L. cutaneous* parasites.[37]

The results of our study were consistent with other studies that used other plants for the treatment of leishmaniasis such as a study conducted by Jarallah and his group[38] who revealed that aqueous and alcoholic extracts of *Nigella sativa* have antileishmanial effects against *L. major* promastigote *in vitro*, a study conducted by Fattahi Bafghi and his group[38] indicated that *Nigella sativa* extract can inhibit parasite growth and viability, which can be useful in the treatment of leishmaniasis. In a study carried out on the antileishmanial activity of *Lavandula angustifolia* on *L. major*, the researchers evaluated that lavender significantly decreased the number of parasites compared with the control.[39]

In addition, Kheirandish *et al.*[40] showed that olive leaf extract has potent therapeutic effects on CL. In the case of chemotherapy, the concentrations of 7,500 and 10,000 µg/mL completely killed the promastigote stages of CL on the sixth and fifth day of treatment and for both concentrations. The reason for this may be attributed to the synergistic effect of the two drugs used, metronidazole and cotrimoxazole, on the promastigate stages *ex vivo* by affecting the mechanical activity of the parasite and its membrane.

Conclusion

The results of this study demonstrate the clear inhibitory impact of the alcoholic and aqueous extract of the *C.*

longa plant on the growth of the *Leishmania* parasite; the findings clearly show that it can kill these parasites and can therefore be employed as a possible treatment for the infection.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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