Evaluation of *in vitro* antileishmanial activity of curcumin and its derivatives “Gallium curcumin, Indium curcumin and Diacethyle curcumin”

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**Abstract.** – **BACKGROUND AND OBJECTIVES:** Leishmania parasites are intracellular haemoflagellates that infect macrophages of the skin and viscera to produce diseases in their vertebrates hosts. Antileishmania therapy is based on pentavalent antimony compounds which toxicity of these agents and the persistence of side effects are severe. Curcumin was identified to be responsible for most of the biological effects of turmeric. Turmeric plant extracts (curcumin and other derivatives) have anti-inflammatory, anti-arthritic, antioxidant, anti-microbial, antileishmanial, hepato protective, anti-cancer, anti-ulcer and anti diabetic activity.

**MATERIALS AND METHODS:** Stock solutions of curcumin, indium curcumin, diacetylcurcumin and Gallium curcumin were made up in DMSO. From the each stock solution serial dilutions were made with phosphate buffered saline and 100 µl of each prepared concentration was added to each well of 96-well micro plate. All tests were performed in triplicate. Negative control only received RPMI-1640 medium with a parasite density of 106 parasites /ml and the positive control contained varying concentration of standard antileishmania compound, Amphotericine B. MTT solution was prepared as 5 mg/ml in RPMI-1640 and 20 µl of this concentration was added to each well. Antileishmanial effects of test agents and control were evaluated by using the MTT assay.

**RESULTS:** Mean growth inhibition of triplicate for each concentration of test agents and control were measured. The IC₀₀ values for curcumin, gallium curcumin [ga (CUR) 3], indium curcumin [in (CUR)3], Diacethyle Curcumin (DAC ) and Amphotericine B were 38 µg/ml, 32 µg/ml, 26 µg/ml, 52 µg/ml and 20 µg/ml respectively. Indium curcumin [in (CUR) 3] with IC₉₀ values of 26 µg/ml was more effective than other three test agents against Leishmania. Mean growth inhibition of triplicate for Amphotericine B as control drug, was 20 µg/ml.

**CONCLUSIONS:** Indium curcumin and Gallium curcumin complex showed more antileishmanial activity than curcumin and diacetylcurcumin and could be suitable candidates for further investigations.

**Key Words:** Leishmania parasites, Curcumin, antileishmania.

**Introduction**

Leishmania species are intracellular parasitic haemoflagellates that infect macrophages of the skin and viscera to produce diseases in their vertebrates hosts. Three major clinical manifestations of Leishmaniasis are recognised: visceral, cutaneous and muco-cutaneous Leishmaniasis¹. Antileishmania therapy is based on pentavalent antimony compounds. The toxicity of these agents and the persistence of side effects are severe, even after modification of the dose level and the duration of treatment². Curcumin is the active ingredient in the herbal remedy and dietary spice turmeric (Curcuma longa Linn). Curcumin was identified to be responsible for most of the biological effects of turmeric³. *In vitro* curcumin has anti-Leishmania activity⁴. Curcumin has got poor bioavailability but is remarkably well tolerated and does not appear to be toxic to animals or humans even at high doses⁵,⁶. Anti-*Plasmodium falciparum* and anti- *Leishmania major* effects of curcumin have also been reported⁷. Growth of several bacteria like *Streptococcus, Staphylococcus* and *Lactobacillus* could be suppressed by curcumin⁸,⁹. The aqueous extract of turmeric rhizomes has reported the antibacterial effects¹⁰. *In vitro* growth of *Helicobacter pylori* can also be prevented by the use of curcumin. Turmeric plant extract (curcumin and other derivatives) have anti-inflammatory, anti-arthritis-
ic, antioxidant, anti-microbial, anti-leishmanial, hepato protective, anti-cancer, anti-ulcer and anti 
diabetic activity. The objective of the present 
study was to evaluate the anti-Leishmanial activi-
ty of curcumin, indium curcumin, diacetyl cur-
cumin, and Gallium curcumin compared with 
Amphotericine B.

Materials and Methods

Parasite Culture and Test Agents

Leishmania major (MRHO/IR/75/ER) pro-
mastigotes were cultivated in RPMI-1640 (Sig-
a, St. Louis, MO, USA) medium supplement-
ed with 10% fetal calf serum (FCS) (Sigma, St. 
Louis, MO, USA) and 50 mg/ml Ampicillin 
(Sigma, Steinheim, Germany). They were 
grown in bulk at 25°C, culture tubes were cen-
trifuged at 2500 g for 10 min and early log 
phase promastigotes were collected. The para-
sites were washed twice with RPMI-1640 (with-
out FCS) and resuspended in the complete 
medium to achieve a final concentration of 10^6 
parasites/ml. The test was performed in 96-well 
microtitre plates and each plate was filled with 
100 µl of culture medium and the plates were 
incubated at 27°C for 1 h before the test agents 
addition. The test agents in this study were cur-
cumin, indium curcumin [In(CUR)3], diacetyl-
curcumin (DAC) and Gallium curcumin [(Ga 
(cur)_3]. Curcumin (Sigma) was purchased and 
then, DAC, In (CUR)3, and Gallium curcumin 
were prepared as previously described. Stock 
solution of the test agents were made up in DM-
SO (dimethylsulfoxide; Merck, Darmstadt, Ger-
many) to ensure complete solubilization.

From the each stok solution of the test agents 
serial dilutions were made with phosphate 
buffered saline (PBS) and 100 µl of each pre-
pared concentration was added to each well of 
96-well Nunc microtiter plate. All tests were 
performed in triplicate. Negative control only 
received RPMI-1640 medium with a parasite 
density of 10^6 parasites/ml and the positive 
control contained varying concentrations of stan-
dard antileishmania compound, Amphotericine B 
(Sigma, Steinheim, Germany). MTT (Sigma, 
St. Louis, MO, USA) solution was prepared as 
5 mg/ml in RPMI-1640 without phenol red and 
filtered through a 0.2 µm filter and 20 µl of this 
concentration was added to each well and incu-
bated at 25°C for 72 hours. After this incubation 
period and in order to solving the formazan 
crystals, 150 µl of acidic isopropanol was added 
to each well. The plate was read on an ELISA 
reader (Biotech, Highland Park, Winooski, VT, 
USA) using 540 nm as test wavelength and 630 
nm as the reference wavelength.

Statistical Analysis

Data were recorded and analyzed using SPSS 
16.0 software (SPSS Inc., Chicago, IL) and p 
value < 0.05 was considered significant. To compare 
the effects of different concentrations of the test 
agents and Amphotericine B as control drug, sta-
tistical analysis was performed using the non 
parametric Kruskal-Wallis test and Mann-Whit-
ney U test was used to compare the different cur-
cumin concentrations.

Results

Different concentrations of curcumin, indium 
curcumin [In(CUR)3], diacetylcurcumin (DAC) 
and Gallium curcumin [(Ga(cur)_3] were evaluat-
ed for their in vitro effects against the Leishma-
nia major promastigotes. Mean growth inhibition 
of triplicate for each concentration of test agents 
and control were measured. The characterization 
of leishmanicidal activity of curcumin and its de-
rivatives as test agents and Amphotericine B as 
standard drug is summarized in Table I. The IC_{50} 
values for curcumin, gallium curcumin 
[Ga(CUR)3], indium curcumin [In(CUR)3], Di-
acethyle Curcumin (DAC ) and Amphotericine B 
were 38, 32, 26, 52 and 20 µg/ml respectively.

Discussion

Antileishmania therapy is based on pentava-
lent antimony compounds. The toxicity of these 
agents and the persistence of side effects are se-
vere, even after modification of the dose level

<table>
<thead>
<tr>
<th>Agents</th>
<th>Cytotoxicity µg/ml</th>
<th>IC_{50} µg/ml</th>
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<tbody>
<tr>
<td>Curcumin</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Gallium Curcumin [Ga(CUR)3]</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Indium Curcumin [In(CUR)3]</td>
<td>26</td>
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<tr>
<td>Diacethyle Curcumin (DAC)</td>
<td>52</td>
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<tr>
<td>Amphotericine B</td>
<td>20</td>
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and the duration of treatment\textsuperscript{16,17}. These problem regarding the Leishmaniasis treatment necessitate the continued effort to identify new improved antileishmanial drugs. As shown in Table I, indium curcumin [in (CUR) 3] with IC\textsubscript{50} values of 26 µg/ml was more effective than other three test agents against Leishmania promastigotes. The second effective agent against Leishmania was Gallium curcumin [Ga(CUR)\textsubscript{3}] with IC\textsubscript{50} of 32 µg/ml. Curcumin with IC\textsubscript{50} value of 38 µg/ml is the third agent and Diacetylcurcumin (DAC) with 52 µg/ml is the fourth agent against Leishmania promastigotes, mean growth inhibition of triplicate for Amphotericine B as control drug, was 20 µg/ml.

Conclusions

Indium curcumin and Gallium curcumin complex have more anti-Leishmanial activity than curcumin and diacetylcurcumin and could be suitable candidates for further in vivo investigations. This results is in accordance with Mohammadi et al study\textsuperscript{12} which shows that the Gallium and indium complexes of curcumin had much lower IC\textsubscript{50} values of cytotoxicity in mouse lymphoma cells.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

References