Abstract. – BACKGROUND AND OBJECTIVES: Leishmania parasites are intracellular haemoflagellates that infect macrophages of the skin and viscera to produce diseases in their vertebrate hosts. Antileishmanial 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-arthritis, 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 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 10^6 parasites/ml and the positive control contained varying concentrations of standard antileishmanial 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 IC50 values for curcumin, gallium curcumin [Ga (CUR) 3], indium curcumin [In (CUR) 3], Diacetyl 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 IC50 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, antileishmanial.

Introduction

Leishmania species are intracellular parasitic haemoflagellates that infect macrophages of the skin and viscera to produce diseases in their vertebrate hosts. Three major clinical manifestations of Leishmaniasis are recognised: visceral, cutaneous and mucocutaneous Leishmaniasis. Antileishmanial 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 treatment. 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 turmeric. In vitro curcumin has anti-Leishmania activity. Curcumin has got poor bioavailability but is remarkably well tolerated and does not appear to be toxic to animals or humans even at high doses. Anti-Plasmodium falciparum and anti- Leishmania major effects of curcumin have also been reported. Growth of several bacteria like Streptococcus, Staphylococcus and Lactobacillus could be suppressed by curcumin. The aqueous extract of turmeric rhizomes has reported the antibacterial effects. 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, hepatoprotective, anti-cancer, anti-ulcer and anti diabetic activity. The objective of the present study was to evaluate the anti-Leishmanial activity of curcumin, indium curcumin, diacetyl curcumin, and Gallium curcumin compared with Amphotericine B.

**Materials and Methods**

**Parasite Culture and Test Agents**

Leishmania major (MRHO/IR/75/ER) promastigotes were cultivated in RPMI-1640 (Sigma, St. Louis, MO, USA) medium supplemented 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 centrifuged at 2500 g for 10 min and early log phase promastigotes were collected. The parasites were washed twice with RPMI-1640 (without 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 well 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 curcumin, indium curcumin [In(CUR)3], diacetylcurcumin (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 DMSO (dimethylsulfoxide; Merck, Darmstadt, Germany) to ensure complete solubilization.

From the each stock solution of the test agents serial dilutions were made with phosphate buffered saline (PBS) and 100 µl of each prepared 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 standard 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 incubated 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, statistical analysis was performed using the non parametric Kruskal-Wallis test and Mann-Whitney U test was used to compare the different curcumin concentrations.

**Results**

Different concentrations of curcumin, indium curcumin [In(CUR)3], diacetylcurcumin (DAC) and Gallium curcumin [(Ga(cur)3)] were evaluated for their *in vitro* effects against the Leishmania 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 derivatives as test agents and Amphotericine B as standard drug is summarized in Table I. The IC50 values for curcumin, gallium curcumin [Ga(CUR)3], indium curcumin [In(CUR)3], Diacethyle Curcumin (DAC) and Amphotericine B were 38, 32, 26, 52 and 20 µg/ml respectively.

**Discussion**

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 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 \text{[in (CUR) 3]} with $IC_{50}$ 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 \text{[Ga(CUR)$_3$]} with $IC_{50}$ of 32 µg/ml. Curcumin with $IC_{50}$ value of 38 µg/ml is the third agent and Diacethyle Curcumin \text{(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 \textit{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 $IC_{50}$ values of cytotoxicity in mouse lymphoma cells.

Acknowledgements

We would like to thank the Vice-chancellor of Research of Bushehr University of Medical Sciences for his financial support.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References


7) ARAJJO CAC, LEON LL. Biological activities of Curcuma longa L. Mem Inst Oswaldo Cruz 2001; 96: 723-728.


