In-Vitro Evaluation of the Anti-Leishmanial Activity of Euphorbia Helioscopia Stem Extract In Comparison With Synthetic Drug Amphotericin B

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ABSTRACT

Behind malaria and filariosis, leishmaniasis is the third largest infectious diseases transmitted by vectors. Aim of this research work was to evaluate the anti leishmanial activity of Euphorbia helioscopia. This research work was done in the Department of Animal Sciences, Parasitology Laboratory, Quaid-i-azam University, Islamabad, Pakistan. The experiment was performed according to the protocol described previously with a slight modification. The results show that the E. helioscopia has anti leishmanial activity with LC₅₀ value ≤ 10 ug/ml. The standard error for the test plant extract and positive control was calculated with 95% confidence interval having significance value of 0.00. Chi square test and Phrobit analysis were done using the SPSS version 21.

Keywords: Antileishmanial activities, Leishmaniasis, Euphorbia helioscopia, Amphotericin B

INTRODUCTION

Behind malaria and filariosis, leishmaniasis is the third largest infectious diseases transmitted by vectors. Leishmaniasis is a term referred to a number of clinical symptoms caused by several species of Leishmania, a protozoan (Zahir et al., 2012). These parasites belong to the genus Leishmania and are transmitted by the bite of a female phlebotomine sand fly (Bero et al., 2011). It is one of the major health problems causing significant morbidity and mortality in Asia, Africa and Latin America (Tahir et al., 1998). There are three clinical forms of leishmaniasis; Cutaneous leishmaniasis (CL), Mucocutaneous leishmaniasis, visceral leishmaniasis (VL), among which Cutaneous leishmaniasis (CL), is the most common form and Visceral leishmaniasis (VL) is the most fatal one with an estimated annual incidence of 500,000 in 61 countries. Several drugs are used to treat leishmaniasis but due to drug resistant strains and side effect cause the search for effective natural drug (Jaffary, 2012; Ullah et al., 2013). Due to variable efficacy, resistant strains and species, the search of new traditional plants having antileishmanial activity is led (Bero et al., 2011).

Some chemical compounds extracted from Leguminosae members showed anti leishmanial activity against Leishmania amazonensis (Araujo et al., 1998). Extracts of Annona muricata were found to be effective against L. Braziliensis and L. panamensis (Jaramillo et al., 2000). Some Diterpenes and triterpenes isolated from Lamiaceae family member Salvia cilicica was reported to have appreciable in vitro antileishmanial activity against L. donovani and L. major (Tan et al., 2002). Rhazya stricta Decne leaves extracts were reported to have Antileishmanial activity against L. major (Khan et al., 2012). Moreover sterols isolated from
the roots of *Pentalinon andrieuxii* are found to have anti-leishmanial activity (Pan *et al.*, 2012).

The genus *Euphorbia* is the largest in Euphorbiaceae or spurge family with more than 2000 species growing in the form of laticiferous herbs, shrubs and trees, in tropical and temperate zones Asia and other parts of the world (Majid *et al.*, 2010). *Euphorbia* species having biological activities like anti-tumor and anti-cancer (Chem, 2006). For decades *Euphorbia* plant materials have been known poisonous to humans and animals. However pharmacological investigation of the genus revealed that its latex has antiviral, anti-cancer, anti-bacterial, cytotoxic and anti-leishmanial properties (Majid *et al.*, 2010).

**MATERIALS AND METHOD**

Aim of the current study was to explore the anti-leishmanial activity of the stem extract of *Euphorbia helioscopia*. The present research was done in the Department of Animal Sciences, Parasitology Laboratory, Quaid-i-azam University, Islamabad, Pakistan.

**Plant Collection**

*Euphorbia helioscopia* (Sun spurge) plants were collected from the vicinity of Quaid-i-azam University, Islamabad, Pakistan. These plants were washed with running water and were identified by Department of Plant Sciences, Quaid-i-azam University, Islamabad. A voucher specimen (QAU/PS/AS256) was kept for future research. Leaves, stem, flowers and roots of the plants were separated and were dried in shade for about three weeks. The stem part was then powdered by electric blender.

**Preparation of Plant Extract**

Thirty grams fine powder of the *Euphorbia helioscopia* stem part was used for extraction in 300 ml of water for 6 hours using Soxhlet apparatus. Following extraction, solvent was removed using Rotary evaporator at lowest temperature of 37°C. Crude extract obtained was collected and stored at 4°C.

**Anti-Leishmanial Assay Procedure**

In vitro antileishmanial activity of experimental plant was performed according to the protocol described by Nabi *et al.* (2012) with a slight modification.

**Parasite Culture**

Promastigotes of *L*. tropica (KWH 23) strains were used for the assay. The strains were incubated at 24°C at standard laboratory condition till a culture of 1x10^6 /ml promastigotes was achieved.

**Preparation of Stock Solution and Dilutions**

A stock solution of 10,000 ppm was prepared from the crude extract by dissolving 1mg of the crude extract in 1 ml Dimethyl sulfoxide (DMSO). The first row of the plate contains 196 µl of M199 media while the remaining rows contain 180 µl of media. About 4 µl of compounds was added to the first row and serially diluted by 10 % and discard 20 µl from the last row.

**Evaluation of Anti-Promastigotes Activity**

The concentration of DMSO was observed not to exceed >5% so that it has no effect on *Leishmania* parasite morphology (1). For maintaining positive and negative control Amphotericin B and DMSO were used respectively. The plate was then incubated at 24°C for 72 hrs. After three days, the mortality was recorded by counting the live promastigotes in neubar counting chamber.
Statistical Analysis

Experiment was carried in triplicates. The average mortality was subjected to probit analysis for calculating LC$_{50}$ at 95% confidence limit using SPSS version 21.

RESULTS

Different concentration of the plant stem extract was taken in comparison with the amphoteracin B as a positive control and 5% DMSO as a negative control. The concentration of the E. helioscopia and amphoteracin B were kept same i.e. 0.05, 0.5, 5, 50, and 500ug/ml. The percent mortality recorded were 4.66±0.76, 10.96±1.05, 29.62±0.86, 60.47±1.44 and 100±0.00 for the aqueous stem extract of E. helioscopia and 30.27±0.54, 45.12±1.10, 80.87±1.78, 100±0.00 and 100±0.00 for amphoteracin B respectively. The mortality in the negative control was zero. The LC$_{50}$ and LC$_{90}$ for E. helioscopia (9.94 ug/ml, 226.54 ug/ml) and amphoteracin B (0.35 ug/ml, 9.49 ug/ml) were also calculated. The chi square ($R^2$) value for the test plant and positive control show negligible differences having value of 0.927 and 0.922, as shown in table 1.

The standard error for the test plant extract and positive control was calculated with 95% confidence interval having significance value of 0.00, and represented with standard error bars in figure 1. The comparative percent mortality of E. helioscopia and amphoteracin B were also shown with different colors along with the trend lines.

Table 1. In vitro anti leishmanial activity of E. helioscopia stem extract, showing average, LC$_{50}$, LC$_{90}$ Values

<table>
<thead>
<tr>
<th>Concentration in (µg/ml)</th>
<th>Anti leishmanial activity of Euphorbia helioscopia</th>
<th>E. helioscopia</th>
<th>Amp B (Positive control)</th>
<th>Negative Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anti leishmanial activity</td>
<td>0.05 (µg/ml)</td>
<td>0.5 (µg/ml)</td>
<td>5 (µg/ml)</td>
</tr>
<tr>
<td>E. helioscopia</td>
<td></td>
<td>4.66±0.76</td>
<td>10.96±1.05</td>
<td>29.63±0.86</td>
</tr>
<tr>
<td>Amp B (Positive control)</td>
<td></td>
<td>30.27±0.54</td>
<td>45.12±1.10</td>
<td>80.87±1.78</td>
</tr>
<tr>
<td>Negative Control</td>
<td></td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Figure 1. In-vitro anti leishmanial activity of E. helioscopia stem extract
DISCUSSION

Over 100 plants have been reported to be active against various forms of leishmanial parasites (Rocha et al., 2005). The other studies showed that the *Ixora coccinea* leaf extract having anti leishmanial activity against the promastigotes of *L. donovani* (Naskar et al., 2013). The root extract of *Perovskia abrotanoides* shows anti leishmanial activities against the *L. major* (Jaafari et al., 2007). The pharmacological screening of methanolic extract of *Aloe vera* leaf and *Tamarix aphylla* bark were assessed to investigate the in vitro anti leishmanial activity of the medicinal plants against cutaneous leishmaniasis by Iqbal et al., (2012).

Their finding show that, *Aloe vera* and *Tamarix apylla* had a significant dose dependant anti promatigote activity against *L. tropica* as that suggest promising phytotherapeutic agents for cutaneous leishmaniasis. The present study showed the anti leishmanial activity of the aqueous extract of *E. helioscopa* on the promastigotes of *L. tropica* (KWH 23) strains. The finding also revealed that, extract of *E. helioscopa* has anti promastigote activity against *L. tropica*. Both studies show that, the different plants used in the research having anti leishmanial activities.

Other members of Euphorbiacea family are reported to have anti leishmanial, antioxidant, larvicidal and insecticidal activities (Zahir et al., 2012). In vivo anti leishmanial effects of traditional herbal extracts against Cutaneous Leishmaniosis was studied by Mohammad, 2011. It is also found that members of the genus Euphorbia have anticancer, anti-proliferative, antimicrobial, anti-inflammatory, anti-helminthic, cytotoxic and antioxidant properties (Serkan Ozbelgin, 2012). The current study showed the anti leishmanial activity of the aqueous extract of *E. helioscopya* on KWH strain (LC 50 = 9.94 ug/ml).

CONCLUSION

The current study revealed that the stem extract of *E. helioscopa* have anti leishmanial activity. The current investigation reveals that *E. helioscopa* extract contain secondary metabolites and other members are used traditionally for the ailment of different diseases, posses’ activity against *Lesihmania tropica*. As crude form of *E. helioscopa* extract showed promising antileishmanial results. *E. helioscopa* and other members need further investigation so that the pure bioactive antileishmanial compounds should be isolated with promising results, less side effects and cost effective.

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REFERENCES


