IN-VITRO ANTHELMINTIC & CYTOTOXIC POTENTIAL OF DIFFERENT EXTRACTS OF CALOTROPIS PROCERA LEAVES

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Received: 29 March 2012, Revised and Accepted: 07 November 2012

ABSTRACT

Different extracts of Calotropis procera leaves were evaluated for in-vitro anthelmintic activity against Indian earthworms Pheretima posthuma, and for in-vitro cytotoxic activity against the Hep-2 (human larynx epithelial carcinoma) cell line. Dose dependent activity was observed in different extracts of plant leaves. It was observed that 70% hydroethanolic extract of Calotropis procera leaves exhibited better in-vitro anthelmintic activity as compared to n-butanol and chloroform extract of Calotropis procera leaves. The results of in-vitro cytotoxic activity suggested that the n-butanol extract had most pronounced cytotoxicity against the Hep-2. Further investigations are required to obtain the clinically important lead molecules for the drug development.

Keywords: Calotropis procera leaves; Traditional medicines; Anthelmintic activity; Cytotoxic activity; SRB Assay; Human larynx epithelial carcinoma.

INTRODUCTION

Calotropis procera (Ait.) R.Br. (giant milkweed) belong to the family Asclepiadaceae, locally known as “aak” is being used as herbal medicine by people living in desert areas and also found sculptured on Shiva temple symbolizing mythological cum medicinal value enjoyed by the plant in ancient India. In the traditional Indian Medicinal system, this plant has been used for various disease conditions including asthma, cold, cough, piles, ulcers, diarrhoea, heart diseases, leprosy, rheumatism and diseases of skin, spleen, liver and abdomen. The leaf of Calotropis procera is sub-sessile, 6-15 cm by 4.5-8 cm, broadly ovate, ovate-oblong, elliptic or obovate acute, pubescent, when young and glabrous on both sides on maturity. The lamina which is dorsiventral with mesophyll differentiated into a palisade and spongy tissue. Extracts from this plant have been found to possess various pharmacological activities. The flowers as well as latex of the plant have been claimed to be useful as an anthelmintic and latex as well as roots of the plant have also been reported to possess cytotoxic activity. The present study was undertaken to find the unexplored anthelmintic and cytotoxic potential of the different extracts of Calotropis procera leaves.

MATERIAL AND METHODS

Plant material

Fresh leaves were collected from the Calotropis procera plant growing in medicinal garden of Rajiv Academy for Pharmacy, Mathura, U.P., India. The plant specimens were authenticated and voucher specimen is NISCAIR/RHMD/Consult/2008-09/1144/176 for future reference. The leaves were cleaned by washing with running water and shade dried and then milled to coarse powder by mechanical grinder.

Preparation of the leaves extracts

The leaf powder (2.0 kg) was extracted by maceration for seven days with 70% ethanol (3 x 2.5 liters) at room temperature and the combined ethanolic extract was evaporated under reduced pressure to yield hydroethanolic fraction as brownish green viscous residue (60 g). The mark was further extracted sequentially by maceration with n-butanol (3 x 2.5 liters) and chloroform (3 x 2.5 liters). The organic layers were evaporated under reduced pressure to yield n-butanol fraction (32 g) and chloroform fraction (19 g). The prepared organic layers were evaporated under reduced pressure to yield hydroethanolic fraction as brownish green viscous residue (60 g). The mark was further extracted sequentially by maceration with n-butanol (3 x 2.5 liters) and chloroform (3 x 2.5 liters). The organic layers were evaporated under reduced pressure to yield n-butanol fraction (32 g) and chloroform fraction (19 g). The prepared extracts were kept under refrigeration for screening of anthelmintic activity.

In-vitro anthelmintic assay

The in-vitro anthelmintic activity was evaluated on adult Indian earthworm Pheretima posthuma due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. The earthworms were collected and washed with normal saline. The earthworms used were of 5 to 6 cm in length and 0.2-0.3 cm in widths for experiment protocol with slight modifications. Five groups of equal size Indian earthworm consisting of six earthworms in each group were released into 50 mL of desired formulation. Each group was treated with one of the following: Vehicle (0.9% w/v NaCl), piperazine citrate (15 mg/mL), and different extracts of (50 mg/mL, 25 mg/mL, 12.5 mg/mL) in normal saline. Observations were made for the paralysis time and subsequently for death time of the worms. The mean paralysis and/or death time for each group was recorded. The time taken by the worms to become motionless, considered as paralysis time, was recorded and the lethal time was also recorded by observing the time taken to become motionless on application of external stimuli by pricking with pin. Piperazine citrate (15 mg/mL) was taken as reference drug. (Table 1 and 3)

Table 1: In-vitro anthelmintic activity of 70% hydroethanolic extract of Calotropis procera leaves

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (mg/ml)</th>
<th>Paralysis Time (min.)</th>
<th>Death Time (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piperazine citrate</td>
<td>15.0</td>
<td>13.3±0.360</td>
<td>31.1±0.230</td>
</tr>
<tr>
<td>70% Hydroethanolic Extract</td>
<td>50.0</td>
<td>5.52±0.143</td>
<td>12.5±0.232</td>
</tr>
<tr>
<td>70% Hydroethanolic Extract</td>
<td>25.0</td>
<td>13.53±0.704</td>
<td>27.50±0.207</td>
</tr>
<tr>
<td>70% Hydroethanolic Extract</td>
<td>12.50</td>
<td>18.58±0.64</td>
<td>29.05±0.628</td>
</tr>
</tbody>
</table>
In vitro cytotoxic assay

Cell line and cell culture

The Human larynx epithelial carcinoma cell line (Hep-2) was obtained from National Centre for Cell Sciences, Pune. Hep-2 cells were grown in Earl’s Minimal essential medium supplemented with 2 mM L-glutamine, 10% Fetal Bovine Serum, penicillin (100 µg/mL), streptomycin (100 µg/mL) and amphotericin B (5 µg/mL) and the cells were maintained at 37°C in a humidified atmosphere with 5% CO₂ and were sub-culture twice a week.

SRB assay

The in vitro cytotoxicity of different extracts of Calotropis procera leaves was determined against Hep-2 using sulforhodamine B (SRB) dye. The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10⁵ cell/mL using medium (MEM) containing 10% new born calf serum. To each well of the 96 well microtitre plate, 0.1 mL of diluted cell suspension (approximately 10,000 cells) was added and kept for 24 hours and incubated at 37°C for 3 days in 5% CO₂ atmosphere for cell monolayer formation. After 24 hours, when a partial monolayer was formed at the bottom of the well, the supernatant was flicked off, washed the monolayer once and 100 µL of different isolated extracts (70% hydroethanolic, chloroform and n-butanol) were added to the culture (cell) in microtitre plates. The plates were then incubated at 37°C for 3 days in 5% CO₂ atmosphere and microscopic examination was carried out and observations recorded every 24 hrs. After 72 hrs, 25 µL of 50% trichloro acetic acid was added to the wells gently such that it forms a thin layer over the drug dilution to form an overall concentration of 10%. The plates were incubated at 4°C for one hour. After that, plates were flicked and washed five times with tap water to remove traces of medium, drug, and serum, and were then air-dried. The air-dried plates were stained with 100 mL of SRB and kept for 30 minutes at room temperature. The unbound dye was then removed by rapidly washing four times with 1% acetic acid. The plates were then air-dried. 100 µL of 10 mM tris base was then added to the wells to solubilize the dye. The plates were shaken vigorously for 5 min. The absorbance was measured using micro plate reader at a wavelength of 540 nm. Suitable blanks and positive controls were also determined. Values of CTC of obtained are summarized in table 4, which is the concentration at which 50% of the cells are dead after 72 hrs of drug exposure.

<p>| Table 2: In-vitro anthelmintic activity of n-butanol extract of Calotropis procera leaves |</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (mg/ml)</th>
<th>Paralysis Time (min.)</th>
<th>Death Time (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Piperazine citrate</td>
<td>15.0</td>
<td>13.32±0.360</td>
<td>31.10±0.230</td>
</tr>
<tr>
<td>n-Butanol extract</td>
<td>50.0</td>
<td>6.3±0.26</td>
<td>14.25±0.12</td>
</tr>
<tr>
<td>n-Butanol extract</td>
<td>25.0</td>
<td>16.03±0.20</td>
<td>29.52±0.61</td>
</tr>
<tr>
<td>n-Butanol extract</td>
<td>12.5</td>
<td>21.03±0.22</td>
<td>48.26±0.16</td>
</tr>
</tbody>
</table>

Results expressed as ±SEM of six worms in each group.

Statistical analysis

The values are expressed as mean ± SE (n=6) and the statistical analysis was performed by Student’s t test. P < 0.05 was considered significant.

RESULTS

In the present work, three different extracts, 70% hydroethanolic, chloroform and n-butanol from leaves of Calotropis procera were used to evaluate in vitro anthelmintic activity against Indian earthworms Pheretima posthuma, and in vitro cytotoxic activity against the Hep-2 (human larynx epithelial carcinoma) cell line.

In-vitro anthelmintic assay

The perusal of the anthelmintic activity data reveals that 70% hydroethanolic extract at the concentration of 12.5 mg/mL showed paralysis and death in 18.58 and 29.05 min. respectively. Similarly the n-butanol extract and chloroform extract at the concentration of 12.5 mg/mL showed both paralysis and death in 21.03 and 48.26 min. & 26.53 and 51.25 min. respectively. The effect increased with concentration.

In-vitro cytotoxic assay

The in-vitro cytotoxic study results showed that the CTC₅₀ value for the three extracts viz., 70% hydroethanolic extract, chloroform extract and n-butanol extract was 170, 110 and 3.7 µg/mL, respectively against the Hep-2 (human larynx epithelial carcinoma) cell line. Thus, the n-butanol extract had most pronounced in-vitro cytotoxicity against the Hep-2.

DISCUSSION

It was observed that 70% hydroethanolic extract showed better activity as compared to n-butanol and chloroform extract of Calotropis procera leaves and reference control piperazine citrate. Different extracts caused paralysis followed by death of the worms at all tested dose levels. The potency of the extract was found inversely proportional to the time taken for paralysis or death of worms. The activity confirms the dose dependent nature of extract.

The n-butanol extract of Calotropis procera leaves exhibited a strong cytotoxicity for Hep-2 cell lines. Chemical constituents reported from the extracts of leaves are alkaloids, flavonoids, tannins, steroids, saponins and glycosides. Three cytotoxic chemical constituents reported are calotropin, uscarin and calotoxin. These biologically active compounds may be responsible for the in-vitro cytotoxic activity of n-butanol extract against the Hep-2 cell lines. Further isolation and identification of the active compounds as lead in the crude extracts is recommended for the drug development.

CONCLUSION

In conclusion the 70% hydroethanolic extract of Calotropis procera leaves had significant anthelmintic activity. The in-vitro cytotoxic study results showed that the n-butanol extract had most pronounced cytotoxicity against the Hep-2 cell line. The results
provide a support for the use of *Calotropis procera* leaves in traditional medicine and suggest its future advance investigation. So, further identification of chemical constituents responsible for these activities as well as pharmacological and toxicological studies and are needed to pinpoint the findings.

**ACKNOWLEDGEMENTS**

Authors acknowledge the support of JSS College of Pharmacy, Tamil Nadu for providing in-vitro cytototoxic assay data. Authors are also grateful to C.P.C.R.I., Kasaragod, Kerala, for providing the earthworms for testing anthelmintic activity.

**REFERENCES**


