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HPLC analysis of harringtonine and homoharringtonine in the needles of Cephalotaxus griffithii alkaloid fraction and cytotoxic activity on chronic myelogenous leukaemia K562 cell

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Harringtonine (HT) and homoharringtonine (HHT) are Cephalotaxus alkaloids with considerable antileukaemic activity. The objectives of this research were to (1) determine the content of HT and HHT present in Cephalotaxus griffithii needles alkaloid fraction (CGAF) and (2) compare the antiproliferative activity of CGAF, with that of HT and HHT on chronic myelogenous leukaemia K562 cell. The concentration of HT and HHT was found to be 122.14 and 16.79 mg/g of CGAF, respectively. Treatment of K562 cells with CGAF, HT and HHT decreased the viable cells in a dose- and time-dependent manner. Interestingly, the maximum cell death was found in CGAF, with IC$_{50}$ value which was 3- to 4.6-fold lower than those of HT and HHT. Our results indicate that HT content in the needles of C. griffithii is higher than HHT, and alkaloids other than HT and HHT in CGAF are predominantly responsible for K562 cell death.

Keywords: Cephalotaxus griffithii; harringtonine; homoharringtonine; leukaemia; K562

1. Introduction

Cephalotaxus has been used in traditional Chinese medicine for the treatment of tumours, rheumatism, dyspepsia and abdominal distension, and as an immunosuppressant (Editorial Board of China Herbal 1999; Cisowski et al. 2005; Saeed et al. 2008). The genus Cephalotaxus has received a great level of scientific interest as it contains ingredients with antileukaemic activity, especially alkaloids (Takano et al. 1996; Morita et al. 2005; Abdelkafi & Nay 2012). Harringtonine (HT) and homoharringtonine (HHT) are a family of cephalotaxine alkaloids synthesised by the genus Cephalotaxus with potent antileukaemic activity (Cephalotaxus Research Coordinating Group 1976; Chinese People’s Liberation Army 187th Hospital 1977). Recently, HHT was approved by the United States Food and Drug Administration (USFDA) for the treatment of adult patient with chronic myeloid leukaemia (http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm325895.htm).

Cephalotaxus griffithii Hook. f., commonly known as Griffith’s plum yew, is a shrub or small tree and found up to an altitude of 2000 m. It is distributed in North-East India, western Sichuan province in China and Myanmar (Shankar 2008). So far, only one study from C. griffithii alkaloids has been attempted. Spencer et al. (1976) identified eight alkaloids including HT and HHT, and determined the alkaloid percentage of crude alkaloid mixture by gas
chromatography–mass spectrometry. However, the individual alkaloid percentage was determined from the total alkaloid content and did not represent the true picture of the actual individual alkaloid percentage present in the crude alkaloid mixture or plant.

The objectives of this research were to (1) determine the content of HT and HHT present in C. griffithii needle alkaloid fraction (CGAF) or C. griffithii needle and (2) compare antiproliferative activity of CGAF, with that of HT and HHT on human chronic myelogenous leukaemia K562 cell.

2. Results and discussion

2.1. Quantification of HT and HHT in CGAF

The presence of HT and HHT was reported (Spencer et al. 1976); however, their actual percentage content present in the crude alkaloid mixture or plant was not determined. The typical HPLC chromatograms of HT and HHT standard, CGAF and CGAF spiked with standards are shown in Figure S1. HT and HHT were eluted in approximately 11.7 and 14.8 min, respectively. Besides HT and HHT peaks, six other distinct peaks corresponding to retention times 7.2, 8.72, 9.3, 9.9, 13.7 and 15.7 min were also observed (Figure S1).

Based on the linear regression equation \(y = 523.43x + 1211.3; \ r^2 = 0.9953\) for HT and \(y = 470.43x - 48.871; \ r^2 = 0.9891\) for HHT) the concentration of HT and HHT was found to be 122.14 ± 7.84 and 16.79 ± 1.69 mg/g of CGAF, respectively. In other words, the percentage contents of HT and HHT in C. griffithii needles were 0.0066% and 0.0009%, respectively. The concentration of HHT was 7.27-fold less than that of HT. The HT and HHT contents in C. griffithii needles were compared with that in other Cephalotaxus spp. (Table 1), and the HT and HHT in C. griffithii was found to be within the range of those observed in Cephalotaxus fortunei and Cephalotaxus sinensis (Yan-Qing et al. 1984). The range of HT and HHT contents in C. fortunei and C. sinensis was obtained by analysing the samples collected in different months, and it was reported that the HT and HHT contents are higher during summer and lower in winter (Yan-Qing et al. 1984). In our study, the contents of HT and HHT were determined in C. griffithii needles collected during winter (January) and were found to be higher than their content in C. sinensis needles collected during winter. However, the contents of HT and HHT in Cephalotaxus harringtonia reported by Choi et al. (2003) are very high and need re-evaluation (Table 1). As reported by Choi et al. (2003), the amounts of cephalotaxine, HT, HHT and isoharringtonine in C. harringtonia needles were 307.8, 304.5, 304.9 and 459.7 mg/g, respectively. If we sum up the contents of all the four alkaloids, the total content is approximately 1374 mg (1.374 g) in 1000 mg (1 g) of C. harringtonia needles which cannot be true.

2.2. Effects of CGAF, HT and HHT on K562 cell

The effect of HT, HHT, CGAF and vincristine (standard control) on K562 cells after treatment with graded doses for 24 and 48 h is shown in Figure 1. The percentage of surviving cells

<table>
<thead>
<tr>
<th>Name of the plant</th>
<th>HT (%)</th>
<th>HHT (%)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. fortunei</td>
<td>0.0048–0.0196</td>
<td>0.0044–0.014</td>
<td>Yan-Qing et al. (1984)</td>
</tr>
<tr>
<td></td>
<td>(0.048–0.196)</td>
<td>(0.044–0.14)</td>
<td></td>
</tr>
<tr>
<td>C. sinensis</td>
<td>0.0008–0.0081</td>
<td>0.0004–0.0232</td>
<td>Yan-Qing et al. (1984)</td>
</tr>
<tr>
<td></td>
<td>(0.008–0.081)</td>
<td>(0.004–0.232)</td>
<td></td>
</tr>
<tr>
<td>C. harringtonia</td>
<td>(304.5)</td>
<td>(304.9)</td>
<td>Choi et al. (2003)</td>
</tr>
<tr>
<td>C. griffithii</td>
<td>0.0066</td>
<td>0.0009</td>
<td>Present study</td>
</tr>
<tr>
<td></td>
<td>(0.06)</td>
<td>(0.009)</td>
<td></td>
</tr>
</tbody>
</table>

Note: Data in parenthesis are expressed in mg/g of needles.
decreased in a dose- and time-dependent manner. The magnitude of effects on the survival of cells varied depending upon the treatments. CGAF treatment induced more K562 cell death than HT and HHT treatments, and IC$_{50}$ value of CGAF was 3- to 4.6-fold lower than those of HT and

![Graph A](image1)

![Graph B](image2)

Figure 1. Viability percent of K562 cells after exposure for (A) 24 h (B) 48 h to six graded doses of CGAF, HT, HHT and vincristine (standard control).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IC$_{50}$ value (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td>CGAF</td>
<td>261.17 ± 14.64$^i$</td>
</tr>
<tr>
<td>HT</td>
<td>798.89 ± 83.08$^i$</td>
</tr>
<tr>
<td>HHT</td>
<td>928.03 ± 115.44$^i$</td>
</tr>
<tr>
<td>Vincristine</td>
<td>6.4 ± 1.3$^i$</td>
</tr>
</tbody>
</table>

Notes: Data expressed as mean ± SD ($n$ = 3). Within a column, means not significantly different from each other share the same superscript letter, while means with different superscript letters are significantly different.
HHT (Table 2). However, vincristine (standard control) was more effective in inducing K562 cell death than CGAF. The antiproliferative effect of HT and HHT on K562 cells was statistically similar (Table 2). In a previous study, Yin et al. (2011) reported that K562 cells were least sensitive to the cytotoxic effect of HHT among six leukaemia cell lines tested.

3. Conclusion

Overall in our study, HT and HHT were found to be less effective in killing K562 cells as compared with CGAF and these results suggest that alkaloids other than HT and HHT in CGAF are predominantly responsible for K562 cell death. These results call for further chemical characterisation of CGAF to isolate the active antileukaemic molecule.

Supplementary material

Experimental details relating to this article are available online, alongside Figure S1.

Acknowledgements

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Competing interest

The authors declare that they have no competing interests.

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