Antiproliferative activity of *Goldfussia psilostachys* ethanolic extract on K562 leukemia cells

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Abstract

A dose-dependent and significant (\(P<0.01\)) antiproliferative effect of *Goldfussia psilostachys* ethanolic extract was observed on K562 cells. The IC\(_{50}\) is ca. 0.5 \(\mu\)g/ml. The extract markedly increases the proportion of cell in G2/M phases and decreases the population of cells in G0/G1 phases. Moreover, the antimitotic effect is correlated with polymerization of microtubule assembly. These results indicate that *G. psilostachys* ethanolic extract inhibits the proliferation of K562 cells and disrupts the normal dynamic of microtubules during mitosis.

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**Keywords:** *Goldfussia psilostachys*; Cell growth inhibition; Cell cycle; Antimitosis; Tubulin polymerization; K562 cell line

1. Introduction

*Goldfussia psilostachys* is a plant grown in the Southwest of China. Extract from *G. psilostachys*, either by water-boiling or by alcohol percolation, may be orally used for the treatment of carbuncle [1]. Moreover, it is topically applied for the treatment of cellulites.
and for the treatment of trauma and snake bite [1]. However, little is investigated about the antiproliferative activity of this plant.

In this paper, we evaluated the antiproliferative effect of *Goldfussia psilostachys* ethanolic extract on leukemia K562 cells.

## 2. Experimental

### 2.1. General

MES: 2-morpholinoethane sulfonic acid, EGTA: ethyleneglycol bis(2-aminoethyl ether)tetraacetic acid, GTP: guanosine triphosphate.

### 2.2. Plant material

*G. psilostachys* whole plants were collected in Xishuangbanna, Yunnan Province, China, in October 1998, and identified by Prof. J.Y. Cui, Xishuangbanna Botany Garten, the Chinese Academy of Sciences. A voucher specimen (Z-17) was deposited in Chengdu Institute of Biology of the Chinese Academy of Sciences.

### 2.3. Preparation of extract

Dried and powdered whole plants (4.1 kg) were percolated with 95% ethanol. After removing ethanol in vacuo; the residue was extracted with ligroin and concentrated in vacuo to give a crude extract (59 g). The crude extract was separated by Si-gel CC eluted with ligroin/acetone (15:1 to 2:1) to afford the extract (1.07 g); $R_f$ 0.10–0.40 on Si-gel TLC developed with ligroin/acetone 3:1.

### 2.4. Animals

New Zealand rabbits of ca. 3 kg were used. They were housed in standard environmental conditions and fed with rabbit diet and water ad libitum.

### 2.5. Cell culture

K562 cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U ml\(^{-1}\) of penicillin and 100 $\mu$g/ml of streptomycin at 37 °C with 5% CO\(_2\).

### 2.6. Isolation of tubulin

Rabbit brain extracts were prepared by electrophoretically homogeneous. The tubulin was purified by repeated cycles of assembly and disassembly as previously described [2,3]. The second cycle assembled pellet was suspended in the ice-cold reassembly buffer (0.1 M MES, 0.5 mM MgCl\(_2\), 1 mM EGTA, pH 6.8) at 5–8 mg/ml. Protein
concentration was determined by the method of Lowry, using bovine serum albumin as standard.

2.7. Cytotoxicity assays

Cells were preincubated in 96-well plates for 24 h and treated with or without *G. psilostachys* ethanolic extract at concentration ranging from 0.01 to 10 mg/ml concentration for 48 h. Paclitaxel, an antitumor drug, was used as control in the assay. The cytotoxicity was evaluated by sulforhodamine B (SRB) protein assay [4]. This colorimetric assay estimates cell number indirectly by staining total cellular protein with the dye SRB. IC$_{50}$ value (the drug concentration required to inhibit cell growth by 50%) was used as a parameter for cytotoxicity.

2.8. Cell staining and flow cytometry analysis

Morphological change analysis was performed by Giemsa staining. The slides were viewed under a light microscope. To record the cells at mitotic stage or mitotic index, at least 1000 cells were counted for each samples. Flow cytometry analysis was performed using the Epics Elite flow cytometer (COULTER, USA) equipped with a 488-nm argon-ion laser. K562 cells were incubated at 37 °C for 0–48 h. After centrifugation, the pellets were washed for two times and then resuspended in 50 μl of PBS. Aliquots containing $1 \times 10^6$ cells/50 μl were fixed in 70% ethanol at $-20$ °C overnight. Cells were stained with PI and kept in dark for 30 min. Each of the samples was analyzed with the Elite 4.0 and DNA Multicycle software.

2.9. Polymerization assays

The tubulin assembly was measured by the standard methods as previously described [5]. The sample containing 2.5 mg of tubulin/ml was incubated with *G. psilostachys* ethanolic extract at 4 °C for 15 min before the addition of 0.5 mM GTP. *G. psilostachys* ethanolic extract was solubilized in DMSO at the concentration of 5 mM. The DMSO was added to polymerization reaction as control. The samples were then warmed to 37 °C in a

![Fig. 1. Effects of *G. psilostachys* ethanolic extract on the growth of K562 cell lines. Cell growth was determined using SRB assay. Data points represent means±S.D. of triplication samples.](image-url)
water bath and polymerization was monitored for 25 min at 350 nm with the spectrophotometer.

3. Results and discussion

As illustrated in Fig. 1, the growth of K562 cells was inhibited by *G. psilostachys* ethanolic extract in a concentration-dependent manner. The growth of K562 cells treated with 1 μg/ml of *G. psilostachys* ethanolic extract for 48 h was completely inhibited. *G. psilostachys* ethanolic extract demonstrated a greater activity on K562 cells with an IC50 value of ca. 0.5 μg/ml.

A morphological change was observed in K562 live cells. We speculated that the antiproliferation effect of *G. psilostachys* ethanolic extract on tumor cells is correlated with antimitotic action. To provide further evidence in support of this hypothesis, K562 cells treated with the extract was examined by Giemsa staining. Most of the untreated cells are normally in interphase, whereas *G. psilostachys* ethanolic extract-treated cells at the concentration of 1 μg/ml are in mitotic stage. The mitotic index was recorded and shown in Fig. 2. The percentage of mitotic cells increases significantly by 8 h. At 24 h, majority of the cells are in mitosis stage. The data show that the antiproliferative effect of *G. psilostachys* ethanolic extract is due to the accumulation of cells in metaphase and correlated well with antimicotic mechanism.

Distribution of cell cycle analysis by flow cytometry also showed that *G. psilostachys* ethanolic extract has very marked effect at 1 μg/ml, which has increased the proportion

Table 1
Effects of *G. psilostachys* ethanolic extract on K562 cell cycle

<table>
<thead>
<tr>
<th>Treatment time (h)</th>
<th>G0/G1 (%)</th>
<th>S (%)</th>
<th>G2/M (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>33.5</td>
<td>56.1</td>
<td>10.4</td>
</tr>
<tr>
<td>8</td>
<td>1.1</td>
<td>31.8</td>
<td>67.0</td>
</tr>
<tr>
<td>24</td>
<td>0.1</td>
<td>10.3</td>
<td>89.6</td>
</tr>
</tbody>
</table>
of cells in the G2/M phase. As reported in Table 1, *G. psilostachis* ethanolic extract-treated cells for 8 h showed a higher population of cells in G2/M and the highest proportion was obtained after 24 h, having a feature consistent with the morphological changes.

To further confirm the mechanism of *G. psilostachis* ethanolic extract arresting cell proliferation in mitosis, we have examined the effect of this extract on tubulin polymerization in vitro. The polymerization was monitored in the absence or presence of 1 μg/ml of *G. psilostachis* ethanolic extract. As reported in Fig. 3, addition of *G. psilostachis* ethanolic extract to tubulin assembled led to rapid polymerization of tubulin. This polymerization is enhanced after 4 min and kept in indicated times. DMSO did not promote assembly in the control reaction. The results obtained here show that *G. psilostachis* ethanolic extract promote the assembly of microtubules. Thereby, *G. psilostachys* ethanolic extract seems to be a potent tubulin polymerization promoter.

The large majority of the antimitotic drugs used in clinic, including vinblastine and Paclitaxel [5,6], are derived from plants. Recently, a compound, combretastatin, isolated from the South African tree *Combretum caffrum*, inhibits mitosis and microtubule assembly [7]. Several antimitotic agents, including other combretastatins, cause microtubule depolymerization, whereas Paclitaxel cause bundling of microtubules by stabilizing them against depolymerization. *G. psilostachis* ethanolic extract promoted the ability of microtubules to assemble in vitro, indicating that its mechanism of action seems to be similar to that of Paclitaxel. Further studies are need to confirm if *G. psilostachys* is a valuable plant for isolate a new promising anticancer candidate.

References