LETTER TO THE EDITOR

Homonojirimycin, an alkaloid from dayflower inhibits the growth of influenza A virus in vitro

G.-B. ZHANG1, B. ZHANG1, X.-X. ZHANG2, F.-H. BING3*

1Pharmacy College, He’nan University of Traditional Chinese Medicine, Zhengzhou 450008, P. R. China; 2Department of Pharmacy, Zhengzhou Railway Vocational & Technical College, Zhengzhou 450052, P. R. China; 3College of Pharmacy, Hubei University of Chinese Medicine, Wuhan 430065, P. R. China

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Summary. – We have previously examined the antiviral effects of total alkaloids from Commelina communis L. (TAC). Here we investigated the active constituents of TAC, responsible for the antiviral effect. Harman, homonojirimycin (HNJ) and 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine were isolated from TAC by HPLC. Only HNJ showed strong antiviral activity against influenza A/PR/8/34 virus (H1N1) as measured by cytopathic effect reduction assay. The results suggest that HNJ is one of the active components of TAC.

Keywords: Commelina communis L.; influenza A virus; alkaloids; homonojirimycin

Commelina communis L. (also known as dayflower) is distributed widely throughout the world. This herb has long been utilized in traditional Chinese medicine for treating noninfectious fever, edema, hordeolum, diabetes, etc (1). Some chemical constituents such as flavonoids, alkaloids, polysaccharides, terpenes as well as sterols have been isolated from this plant (2). We have recently demonstrated that total alkaloids derived from C. communis L. (TAC) could inhibit influenza A virus growth in vitro; TAC administration could significantly increase the survival rate and reduce the lung damage of mice resulting from experimental influenza virus infection (3, 4). The chemical components with antiviral activity, however, have not yet been identified. The objective of the present study was to investigate the biologically active constituents of TAC, responsible for the antiviral effect.

The plant material used in this study, C. communis L., was collected on Tortoise Hill (Hubei, China) and authenticated by Prof. Ke-Li Chen (College of Pharmacy, Hubei University of Chinese Medicine). TAC (6.5 g) were prepared as described in our previous reports (3). Further separation was achieved through preparative HPLC (column: Asahipak NH2P, 4.6 i.d. × 250 mm; eluent: 80% acetonitrile; flow rate: 1.5 m/min; UV detector: 200 nm), producing compound 1 (75 mg), compound 2 (130 mg) and compound 3 (225 mg). The chemical structures of compounds 1, 2 and 3 were, respectively, elucidated as harman, homonojirimycin (HNJ) and 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine (DMDP) by spectroscopic analyses and comparison with those reported in the literature (5, 6). Test solutions were prepared by dissolving the purified components of TAC and positive control, Ribavinin, in DMSO. Test solutions were further diluted to the required concentrations using MEM; the final concentration of DMSO was 0.1%. The purity of all test compounds was greater than 95%.

MDCK cells were maintained in MEM containing 10% fetal bovine serum. The influenza A/PR/8/34 virus (H1N1 subtype), provided by the Institute of Virology, Wuhan University, was propagated in the allantoic cavity of 11-day-old chick embryos. Virus titration was performed by the limiting dilution method.
The principal objective of the present investigation was to examine the antiviral effects of total alkaloids from *C. communis* L. The antiviral activities of alkaloids from *C. communis* L. were determined by CPE reduction method. Briefly, a virus suspension (100 TCID₅₀/0.1 ml) was added to the MDCK cells in a 96-well culture plate. After incubation at 37°C for 2 hr, the virus solution was removed and cells were washed with PBS. Serial two-fold dilutions of the test compounds were dissolved in culture medium and added to each well in quadruplicate. The plates were then incubated in 5% CO₂ incubator for 2 days, 20 μl of MTT (5 mg/ml in cell culture medium) was added to each well and cells were incubated for an additional 3 hr. The absorbance at 570 nm (A₅₇₀) was measured with a microplate reader. Cytotoxicity was expressed as the 50% cytotoxic concentration (CC₅₀) determined using the regression equation obtained by comparing the A₅₇₀ of a treated well with the A₅₇₀ of an untreated well.

The antiviral activities of alkaloids from *C. communis* L. were measured as the selectivity index (SI), the value of the CC₅₀ divided by the EC₅₀. EC₅₀ was calculated as the percentage of inhibition relative to the virus control group. Values were presented as mean ± S.D. Significance of differences was tested by Student’s *t*-test and those at *P* < 0.05 were considered statistically significant.

Plants and natural products are an invaluable source for searching potential antiviral agents. We have previously examined the antiviral effects of total alkaloids from *C. communis* L. (TAC). The principal objective of the present investigation was to provide data about the individual chemical constituents of TAC responsible for its anti-influenza virus activity. The results demonstrated that HNJ possessed strong antiviral activity against influenza virus A/PR/8/34 with an EC₅₀ value of 10.4 μg/ml and SI value of 17.9, respectively, comparable to those of Ribavirin, an approved antiviral drug (Table 1). Harman was less effective in the inhibition of viral replication, with a higher EC₅₀ and lower SI value, which limits its therapeutic potential considerably. DMDP was slightly toxic to MDCK cells with a CC₅₀ value of 94.3 μg/ml (SI < 1), proving that it did not show any activity against influenza virus at the tested concentration. A SI > 4 was considered to indicate a significant selective antiviral effect, and only HNJ showed strong antiviral effect against influenza A/PR/8/34 virus, unlike harman and DMDP. Harman, HNJ and DMDP obtained from *C. communis* L. were first reported by Bae et al. (5). There are no relevant reports on anti-influenza virus activity of harman and HNJ. According to Elbein et al. (8), DMDP isolated from *Lonchocarpus sericeus* did not inhibit influenza virus production, which is in line with our results. Based on these findings, HNJ is suggested to be one of the active components in TAC.

In conclusion, the present study has demonstrated that HNJ inhibits the growth of influenza A virus *in vitro*. It is of interest how HNJ affects virus replication cycle: by a direct inactivation effect of virus infectivity or by the inhibition of viral protein synthesis. Thus, further investigations have to be done. In addition, the therapeutic efficacy of HNJ in influenza virus-induced pneumonia in mice needs to be evaluated.

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### References


### Table 1. Anti-influenza virus activities of alkaloids from *C. communis* L. against A/PR/8/34 (H1N1) in MDCK cells

<table>
<thead>
<tr>
<th>Test drug</th>
<th>CC₅₀ (μg/ml)</th>
<th>EC₅₀ (μg/ml)</th>
<th>SI</th>
<th>&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>harman</td>
<td>240.8</td>
<td>68.9</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>HNJ</td>
<td>186.0</td>
<td>10.4</td>
<td>17.9</td>
<td></td>
</tr>
<tr>
<td>DMDP</td>
<td>94.3</td>
<td>121.5</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>Ribavirin</td>
<td>325.8</td>
<td>15.2</td>
<td>21.4</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>CC₅₀ = 50% cytotoxic concentration; EC₅₀ = 50% effective concentration; SI = selectivity index = CC₅₀/EC₅₀.