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## LETTER TO THE EDITOR

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

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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.

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**Abbreviations:** CPE = cytopathic effect; DMDP = 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine; SI = selectivity index; HNJ = homonojirimycin; TAC = total alkaloids from *Commelina communis* L.

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

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

| Test drug | $CC_{50}$[a] (μg/mL) | $EC_{50}$[b] (μg/mL) | SI[c] |
|---|---|---|---|
| harman | 240.8 | 68.9 | 3.5 |
| HNJ | 186.0 | 10.4 | 17.9 |
| DMDP | 94.3 | 121.5 | <1 |
| Ribavirin | 325.8 | 15.2 | 21.4 |

[a]$CC_{50}$ = 50% cytotoxic concentration; [b]$EC_{50}$ = 50% effective concentration; [c]SI = selectivity index = $CC_{50}/EC_{50}$.

(*7*), using a 96-well culture plate with 6 wells per dilution. The cytopathic effect (CPE) in each well was observed for 4–5 days, and the virus titer was calculated as the 50% tissue-culture-infective dose ($TCID_{50}$). To test for cytotoxicity, MDCK cells ($2 \times 10^4$ cells per well) were seeded onto a 96-well culture plate and incubated overnight. The culture medium was removed and replaced with a medium containing serial two-fold dilutions of the test compounds. After cells were cultured at 37°C in a 5% $CO_2$ 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 additional 3 hr. The absorbance at 570 nm ($A_{570}$) was measured with a microplate reader. Cytotoxicity was expressed as the 50% cytotoxic concentration ($CC_{50}$), determined using the regression equation obtained by comparing the *A* of a treated well with the *A* of an untreated well.

The antiviral activities of alkaloids from *C. communis* L. were determined by CPE reduction method. Briefly, a virus suspension (100 $TCID_{50}$/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% $CO_2$ at 37°C for 1–3 days until CPE corresponding to 100 $TCID_{50}$ was achieved. After removing the culture medium, 20 μl of MTT (5 mg/ml in cell culture medium) was added to each well and cells were incubated for additional 3 hr. $A_{570}$ was measured with a microplate reader. Positive controls with Ribavirin, untreated virus controls and uninfected, untreated cell controls were also included. Results were transformed into percentage of the controls, and finally expressed as the selectivity index (SI), the value of the $CC_{50}$ divided by the 50% effective concentration ($EC_{50}$). $EC_{50}$ 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 $EC_{50}$ 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 $EC_{50}$ and lower SI value, which limits its therapeutic potential considerably. DMDP was slightly toxic to MDCK cells with a $CC_{50}$ 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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