

Isolation, Characterization and Analgesic Activity of Natural Allantoin from *Portulaca oleracea* Seed

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Received date: January 29, 2018; Accepted date: February 13, 2018; Published date: February 19, 2018

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Abstract

The title compound, allantoin was first isolated from the acetyl acetate fraction of *Portulaca oleracea* seed and characterized by ¹H-NMR and ¹³C-NMR. The analgesic activity of allantoin was assessed in acetic acid-induced writhing test in mice with i.p. injection. The results of the research indicated that the analgesic activity of allantoin was similar to that of aspirine. After administrated of allantoin regularly and repeatedly, the mice did not reveal addiction phenomenon. Allantoin could be further studied as a promising analgesic drug.

Keywords: *Portulaca oleracea* seed; Allantoin; Crystal structure; Analgesic activity

Introduction

Portulaca oleracea a common plant distributing in many parts of the world, is a common “weed” in field crops as well as in turf grass. It is a kind of traditional ethno drug herbs as well as a vegetable used for human consumption [1]. *Portulaca oleracea* is recorded in Chinese pharmacopoeia and has detoxifying, heat-clearing, pain-swelling, haemostatic and blood-cooling functions, usually used for the treatment of enteritis and dysentery in clinic [2]. Many modern pharmacological studies have revealed certain pharmacological actions of this herb, such as including antidiabetic, antioxidation, anticancer, anti-hypoxic, anti-inflammatory, neuroprotective, bacteriostatic action and hepatic protection [3]. To find more bioactive compounds, the chemical compositions of *Portulaca oleracea* seed was further investigated and one highly purified compound named allantoin was isolated successfully. The chemical structure of the allantoin was characterized by melt point and NMR analysis. Further, the target compound was also assessed for analgesic activity by acetic acid-induced writhing test.

Materials and Methods

General experimental procedures

The NMR (500 MHz) spectrum was analysed on a Bruker AvanceIII-500 spectrometer and TMS (tetramethylsilane) served as an internal standard. Melting point of the isolated compound was recorded on RD-2 micromelting point apparatus. GF₂₅₄ for TLC and Silica gel (200-300 mesh for Column Chromatography) were purchased from Qingdao Marine Chemical Company (Qingdao, China). Sephadex LH-20 was from Amersham Biosciences (Uppsala, Sweden).

Plant material

Portulaca oleracea seed was gathered in Chuzhou County, Anhui Province, China, in January 2016. The plant was identified by Prof. Jianwei Chen of Nanjing University of Chinese Medicine. A voucher specimen (No.PA160918) has been stored at the Department of Pharmaceutical and fine Chemicals, School of Chemical Engineering, Nanjing University of Science and Technology, Nanjing, China.

Extraction and isolation

Portulaca oleracea seed (100 g) was grinded into fine powder, extracted with ethyl acetate (One liter × 2, 2 h each times) and filtered to obtain filtrate. Ethyl acetate was removed from the filtrate by rotary evaporation and the offwhite extract (10.0 g) was obtained. Ethyl acetate extract was subjected to silica gel chromatography by gradient elution with petroleum (60°C~90°C)-acetone (100:0, 100:1, 100:2, 100:4, 100:8, 100:16, 100:32, 100:100, and 0:100) to afford 90 fractions (F1-F90). F31-F40 (A) was permeated through Sephadex LH-20 by a methyl alcohol-chloroform (1:1) system to give 10 subfractions A1-A10. Fractions A7-A9 were purified with recrystallization in methyl alcohol-water (1:1) system to obtain compound (1) (850 mg).

Compound identification

The isolated compound was easily determined as allantoin by comparing its NMR spectra data with the data reported in literature [4]. Melting point: 234°C~235°C. ¹H-NMR (500 MHz, CDCl₃) δ H=10.55 (1H, s, H-1), 8.06 (1H, s, H-3), 6.90 (1H, d, J=8.15 Hz, H-6), 5.80 (2H, s, H2-8), 5.24 (1H, d, J=8.15 Hz, H-4); ¹³C-NMR (500 MHz, CDCl₃) δ C=174.05 (C-5), 157.82 (C-7), 157.22(C-2), 62.87 (C-4).

Analgesic effect study

Kunming mice of half female and half male (8-22 g) purchased from the animal center of Nanjing University of Chinese Medicine, were used for acetic acid-induced writhing test. Adult mice (20-25 g) were used to study the addiction of the isolated compound. Mice were maintained at 20-25°C under a 12 h light/dark cycle. Standard food

and water were available random. All animal experimental protocols conformed with the Guidelines for Animal experimentation of the university.

Results and Discussion

Compound (1) was readily identified as allantoin by the analysis of their NMR spectra and by the comparison with the data reported in literature [4]. The structure of this compound is given in Figure 1. The 500 MHz ¹H-NMR spectrum clearly defined six protons. The one proton singlets at δ 10.55 and 8.06 were assigned to H-1 and H-3 respectively. The one proton doublet at δ 6.90 (J_{H,H}=8.15 Hz) was attributed to H-6. The two-proton doublet at δ 5.80 was due to the NH₂ group and the one proton doublet at δ 5.24 (J_{H,H}=8.15 Hz) was assigned to H-5. The melting point of the crystals was 234°C~235°C. The crystalline compound was easy to determine as allantoin on the basis of the melting point and the spectral data.

Analgesic activity of allantoin in mice was assessed by writhing responses during a 20 min period, after i.p. 1% solution of acetic acid in physiological saline (0.1 mL/10 g).

Allantoin and aspirin were dissolved in 1% solution of Tween80 in physiological saline.

The solutions of samples and the vehicle of Tween80 were injected by i.p. (0.1 mL/10 g) 15 min prior to acetic acid solution. Analgesic activity (Inhibition) was calculated on the basis of the following equation: % Inhibition=(W₁-W₂/W₁) × 100, Where W₁ is average writes in the vehicle of Tween 80 treated group, W₂ is average writes in the drug-treated group. Statistical significance of the data was performed with analysis of variance (ANOVA) followed by Student's t-test (Table 1).

The results (Table 1) showed that i.p. of allantoin was very effective to prevent abdominal constrictions caused by acetic acid. Allantoin with inhibition of 51.6% is in accord with aspirin at the same dose (20 mg/Kg). Allantoin also produced a dose-dependent proliferation in the mice experiment of abirritation (Figure 2). There was no significant difference in analgesic effect between allantoin and aspirin within 5 mg/Kg to 80 mg/Kg.

The test mice were received s.c. solution of allantoin and morphine (twice a day) with dose showed in Table 2 for seven days. Tween80 (0.1 mL/10 g) was administrated to the control group. Naloxone was s.c. injected at dose of 6 mg/Kg after 6 h of the last solution of allantoin or morphine to produce addiction. The experimental mice were put on a

column of 30 cm diameter and 35 cm high. The number of jumping mice was recorded for 30 min after s.c. naloxone solution (Table 2). It showed that the test mice were not addicted to allantoin at the dose of 120 mg/Kg. It did not reveal addiction phenomenon (appetite drawback tail erection, and reward-seeking behavior etc) in the test allantoin group, but morphine group possessed.

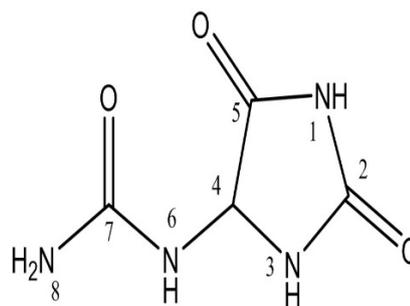


Figure 1: Chemical structure of allantoin.

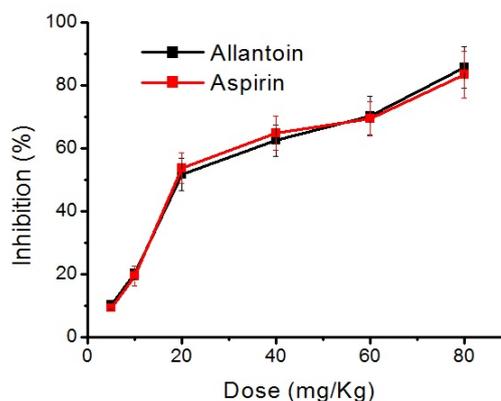


Figure 2: Dose-dependent Analgesic Effect of allantoin.

Compound	Dose/mg.Kg ⁻¹	Mice/n	Writhing/($\bar{X} \pm S$)	Inhibition/%
Tween80	-	10	212.8 ± 10.5	-
Aspirin	20*	10	103.2 ± 10.4*	51.5 ± 5.2
Allantoin	20*	10	104.8 ± 11.6*	51.6 ± 5.7

Table 1: Analgesic activity of Allantoin and other compounds in acetic acid -induced mice Writhing test. *P<0.01 compared with Tween80 group.

Compound	Dose (mg/Kg)	Mice/n	Jumping Mice/n	Rate/%
Tween80	-	20	0	0
Morphine	120	20	18	90

Allantoin	120	20	0	0
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Table 2: Results of addiction of Allantoin in mice.

Conclusion

Allantoin was isolated from *Portulaca oleracea* seeds for the first time. The analgesic effect of allantoin was evaluated in acetic acid-induced abdominal constriction tests in mice with i.p. injection. The results indicated that the analgesic effect of allantoin was almost as strong as aspirine. Allantoin was observed to be analgesics with dose-dependence proliferation and the tested mice did not exhibit a tendency to be addicted to allantoin. These results suggested that allantoin could be as a promising candidate as novel analgesic agent.

References

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