Anti-ulcer Effect in Rats of Bitter Cardamon Constituents

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The effects of bitter cardamon (the fruit of *Alpinia oxphylla*), used as a medicine and a condiment, on HCl/ethanol-induced gastric lesions in rats were examined. The acetone extract at 50 mg/kg, *p.o.* significantly inhibited gastric lesions by 57.0%. An analysis of the active constituents in the acetone extract was performed using column chromatography. Nootkatone at 20 mg/kg, *p.o.* significantly inhibited gastric lesion. These results suggest that nootkatone, the sesquiterpenoid is an important constituent in stomach medications containing bitter cardamon.

Keywords bitter cardamon; anti-ulcer effect; nootkatone; sesquiterpenoid; stomach medication

Introduction

There are few reports describing the stomachic property of bitter cardamon.[1] Bitter cardamon, the fruit of *Alpinia oxyphylla* MIQ. (Zingiberaceae), is a plant cultivated in southern China (Hai Nan Island, Leizhou Peninsula) and used as a stomachic and a condiment.

Screening for the development of new drugs from natural products, acetone extract of bitter cardamon at 300 mg/kg, *p.o.* was found to be effective in treating HCl/ethanol-induced ulcers in rats. Further analysis of the extract indicated strong activity in the fraction containing nootkatone, which was confirmed to be one of the active constituents.

Materials and Methods

Fractionation and Purification Bitter cardamon (2.5 kg), as described in the Japanese Pharmacopea, was purchased from local markets in Osaka and was powdered. Acetone extract of bitter cardamon was obtained as follows:

Five volumes of acetone were added to bitter cardamon for 1 d at room temperature. The same procedure was repeated twice thereafter. The filtered solution was concentrated under reduced pressure below 40 °C and the solvent was completely eliminated. The yield of the extract was 4.7%. The acetone extract was fractionated into 4 fractions by column chromatography using Silica gel 60 with benzene : acetone = 20 : 1 as shown in Fig. 1.

Fractions 1, 2 and 3 were found to be effective in HCl/ethanol-induced ulcers in rats. In the present study, in order to examine the active constituents of fr. 2, it was subjected to silica gel column chromatography (benzene : acetone = 30 : 1) and the products were purified. Comparing the spectra of fr. 2 and the standard indicated that the compound in the fr. 2-2 was nootkatone. The dosage was determined based on the rate of recovery for each of the fractions.

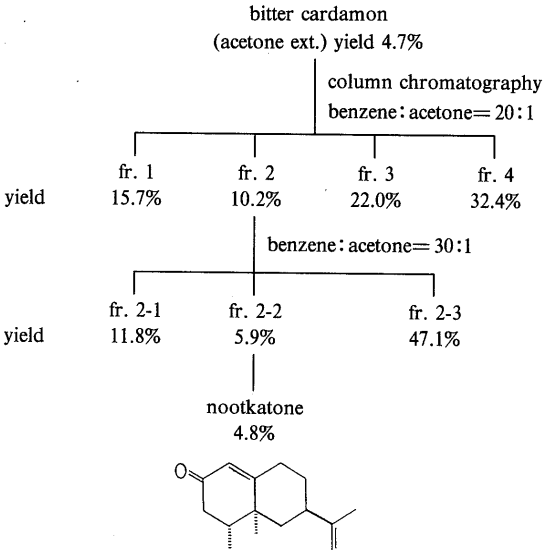

Fig. 1. Fractionation of Bitter Cardamon Acetone Ext.

HCl/Ethanol-Induced Ulcers Ulcers were developed according to the methods of Mizui and Doteuchi.[2] Briefly, after each rat (about 250 g) fasted for 24 h, HCl/ethanol (60% ethanol + 150 mM HCl) at the amount of 1.5 ml/rat was orally administered. One hour thereafter, animals were killed with ether and the stomach was excised. Then 10 ml of 2% formalin was infused into the stomach and the stomach was soaked in 2% formalin for 10 min. The stomach was then cut open along the greater curvature and the length of each lesion in the glandular portion was summed (total length of ulcers) and the lesion index was calculated. Test drugs were administered orally 1 h prior to the administration of HCl–ethanol. Test drugs were suspended in 5% arabic gum and administered orally. Concentrations of the drugs were adjusted so that the volume of administration was 1 ml/200 g of body weight. Cetraxate (Daiichi) was used as a reference drug.

Statistical Analysis Student's *t*-test for HCl/ethanol-induced ulcers were used for statistical analysis.

Results

The acetone extract of bitter cardamon at 50, 150 and 300 mg/kg, *p.o.* each, significantly inhibited gastric mucosal membrane lesions (Table I).

The acetone extract was fractionated into fr. 1 to fr. 4 as shown in Fig. 1. Fractions 2 and 3 significantly inhibited the gastric mucosal membrane lesions at the doses used (Table II).

Fraction 2 was further fractionated, and nootkatone significantly inhibited at 10 mg/kg, *p.o.*, by 43.6% and 20 mg/kg, *p.o.* by 69.8% respectively (Table III).

Discussion

There are many Chinese medicines and natural medicines

TABLE I. Effects of Bitter Cardamon (Acetone Ext.) and Cetraxate on HCl/Ethanol-Induced Gastric Ulcers in Rats

Treatment	Dose (mg/kg)	*N*	Total area of ulcers (mm²)	Inhibition (%)
Control		8	91.6 ± 8.6	—
Bitter cardamon	50	8	39.4 ± 10.3[a)]	57.0
	150	8	0.0 ± 0.0[a)]	100.0
	300	8	0.0 ± 0.0[a)]	100.0
Cetraxate	300	8	29.8 ± 15.4[a)]	84.6

Gastric lesions were induced by oral administration of 1.5 ml of 60% ethanol (v/v) in 150 mM HCl (HCl/ethanol). Each drug was administered orally 1 h before HCl/ethanol treatment. Animals were sacrificied 1 h after HCl/ethanol treatment. Each value represents the mean ± S.E. Significantly different from the control at *a*) $p < 0.01$.

TABLE II. Effects of Fractions of Bitter Cardamon (Acetone Ext.) and Cetraxate on HCl/Ethanol-Induced Gastric Ulcers in Rats

Treatment	Dose (mg/kg)	N	Total area of ulcers (mm^2)	Inhibition (%)
Control		7	95.6±11.9	—
Ext.	300	7	1.3± 0.8[a]	98.6
Fr. 1	50	7	17.1± 9.1[a]	82.1
Fr. 2	30	7	37.4± 7.5[a]	60.9
Fr. 3	70	6	1.6± 1.2[a]	98.3
Fr. 4	100	7	56.2± 8.0[b]	41.2
Cetraxate	300	6	0.4± 0.4[a]	99.6

Gastric lesions were induced by oral administration of 1.5 ml of 60% ethanol (v/v) in 150 mM HCl (HCl/ethanol). Each drug was administered orally 1 h before HCl/ethanol treatment. Animals were sacrificed 1 h after HCl/ethanol treatment. Each value represents the mean±S.E. Significantly different from the control at *a*) $p<0.01$, *b*) $p<0.05$.

TABLE III. Effects of Fractions of Fraction 2 of Bitter Cardamon (Acetone Ext.) and Cetraxate on HCl/Ethanol-Induced Gastric Ulcers in Rats

Treatment	Dose (mg/kg)	N	Total area of ulcers (mm^2)	Inhibition (%)
Control	—	7	111.3± 9.8	—
Fr. 2	60	5	8.8± 6.3[a]	92.1
Fr. 2-1	15	6	54.3±10.8[a]	51.1
Fr. 2-2	10	7	62.6± 6.2[a]	43.6
Nootkatone	20	7	33.5± 6.2[a]	69.8
	50	6	17.8± 3.7[a]	82.8
Fr. 2-3	50	5	7.7± 1.3[a]	93.1
Cetraxate	150	6	36.3± 5.1[a]	67.3

Gastric lesions were induced by oral administration of 1.5 ml of 60% ethanol (v/v) in 150 mM HCl (HCl/ethanol). Each drug was administered orally 1 h before HCl/ethanol treatment. Animals were sacrificed 1 h after HCl/ethanol treatment. Each value represents the mean±S.E. Significantly different from the control at *a*) $p<0.01$.

used for gastrointestinal disorders, which are effective in clinical usage as well. However, very few of them have been examined for their effectiveness in pharmacological experiments. In order to develop new drugs from natural products and to substantinate the clinical actions of bitter cardamon, it appeared useful to examine the effect of bitter cardamon and its constituents on HCl/ethanol-induced gastric lesions in rats. The results in the present experiment indicated that nootkatone obtained at about 4.8% from acetone extract, is one of the active constituents in the HCl/ethanol-induced ulcer model, which has frequently been used to determine the activation of gastric mucosal membrane protective factors. Acetone extract at 50 mg/kg, *p.o.* inhibited the gastric lesions by approximately 60%, but nootkatone significantly inhibited the gastric lesions by approximately 83% at 50 mg/kg. These results indicate the importance of the other ingredients in acetone extract for the anti-ulcer effect. However, despite widespread studies in animals and humans, the pathogenesis of this lesion remains poorly understood, as does the mechanism of its attenuation by cytoprotective agents.[3] It has been reported that prostaglandins (PGs) are involved in the protective action in gastrointestinal cells.[4,5] It is also known that PGE_2 and its derivatives are useful. Furthermore, the 5-lipoxygenase inhibitors, like BW 755C or nordihydroguaiaretic acid, display protective properties in ethanol induced gastric ulcers.[2,6,7] Further experiments are in progress to clarify the mechanism of action of nootkatone and the other active ingredients in acetone extract. Especially, the effects of nootkatone are being investigated on PG synthesis and the 5-lipoxygenase pathway in the gastric mucosal membrane.

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