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Studies on the Origin, Processing and Quality of Crude Drugs. II.[1] Pharmacological Evaluation of the Chinese Crude Drug "Zhu" in Experimental Stomach Ulcer. (2). Inhibitory Effect of Extract of *Atractylodes lancea* on Gastric Secretion

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In order to identify the active principles in the 50% MeOH extract of the Chinese crude drug "Chang Zhu" (consisting of *Atractylodes lancea* rhizome), which has a preventive activity against experimental ulcerations, the effects of the benzene, methanol and aqueous fractions from 50% MeOH extract on gastric secretion in pylorus-ligated rats were investigated.

The terpenes, β-eudesmol and hinesol, contained in benzene fraction were found to be highly effective. β-Eudesmol markedly inhibited ulcers in Shay rats, as well as histamine- and aspirin-induced gastric ulcers, and showed anti-secretory activity on gastric acid secretion stimulated by histamine in a perfused rat stomach preparation.

The anti-ulcerogenic activity of 50% MeOH extract of *Atractylodes lancea* rhizome might be mainly attributable to the H_2-antagonistic effect of β-eudesmol.

Keywords——*Atractylodes lancea* rhizome; histamine-induced ulcer; gastric secretion; aspirin-induced ulcer; Shay ulcer; β-eudesmol; hinesol

"Zhu" is one of the important drugs which are frequently prescribed for the treatment of indigestion and stomach ache in the traditional Chinese system of medicine. In the preceding paper, we reported that 50% methanol extract of "Chang Zhu" (consisting of *Atractylodes lancea* rhizome), among four species of "Zhu" on the market, markedly inhibited ulcers in Shay rats, as well as histamine- and aspirin-induced gastric ulcers, and gastric juice secretion in rats.

In this paper we have examined the benzene, methanol and aqueous fractions prepared from 50% methanol extract of *Atractylodes lancea* rhizome for inhibitory activity on gastric juice secretion in pylorus-ligated rats. We extracted the main active principle and investigated its mode of action in comparison with those of known anti-ulcerogenic agents.

Materials and Methods

Materials——The rhizome of "*Atractylodes lancea*," originating from Sado, but purchased from the market in Osaka, was powdered and extracted twice with 50% methanol for 3 h at 80 °C. The methanol extract was evaporated to dryness *in vacuo* to give a brownish residue. The residue was extracted succesively with benzene, methanol and water, and each extract was tested for activity on gastric juice secretion in pylorus-ligated rats.

The benzene extract was chromatographed on $AgNO_3$–silicic acid using *n*-hexane–AcOEt (100 : 3) as the eluent. Hinesol and β-eudesmol were isolated from the eluate according to the procedures of Yoshioka *et al.*[2]

Animals——Male Wistar-King strain rats weighing 150—220 g and Hartley strain guinea-pigs weighing 300—350 g were used. The animals were housed in a room maintained at 23 ± 1 °C and 60% relative humidity for 7 d before the start of the experiment, with free access to food and water.

Assay of Gastric Secretion Inhibitory Activity in Rats——Gastric secretion inhibitory activity in rats was assayed according to the method of Shay *et al.*[3] Rats were fasted for 48 h, then the pyloric region was ligated under light anesthesia with ether. After 4 h, the rats were again anesthetized with ether and the stomach was removed. The gastric contents were collected in test tubes and centrifuged at 8000 rpm for 10 min. The volumes of gastric juice were measured. Total acid output was titrated with 1/50 N NaOH and total peptic activity was determined according to the method of Anson.[4] Each sample was suspended in 0.2% carboxymethylcellulose (CMC) at the required concentration and administered orally 1 h before or intraduodenally immediately after pylorus ligation.

As a control, 0.2% CMC was administered and atropine sulfate (Tokyo Kasei Industry Co., Ltd.) and cimetidine (Smith Kline & Fujisawa) were used as the positive control drugs.

Anti-ulcerogenic Activity in Rats——Pylorus-Ligated Ulcer (Shay Ulcer): Rats were fasted for 24 h, then the pyloric region was ligated in the same manner as mentioned above. After 16 h, the rats were sacrificed, and the stomach was removed. The gastric mucosa was exposed by opening the stomach along the greater curvature and gastric ulcers of the forestomach were observed. The protective action was evaluated in terms of the ulceration index. The severity of lesions of the forestomach in survivors was classified macroscopically based on an arbitrary scale of 6 grades as reported by Narumi *et al.*[5] A sample or a positive control agent was administered orally 1 h before or intraduodenally immediately after pylorus ligation.

Aspirin-Induced Gastric Lesions: Rats were fasted for 24 h, then according to the method of Okabe *et al.*,[6] they were dosed orally with aspirin (100 mg/kg) suspended in 1% CMC solution immediately after pylorus ligation. After 6 h, the rats were sacrificed and the stomachs were removed, fixed in 1% formalin, and opened. The length of each lesion was measured and the sum was taken as the ulcer index. A sample or a positive control agent was orally administered 1 h before pylorus ligation.

Histamine-Induced Gastric Lesions: Rats were fasted for 48 h, then according to the method of Buchner *et al.*,[7] they were dosed intraperitoneally with histamine hydrochloride (300 mg/kg). After 4 h, the rats were sacrificed, and the stomach was removed and opened. The ulcer index was obtained by the method of Adami *et al.*[8] from the sum of the area of each lesion in the glandular portion. A sample or a positive control agent was orally administered 1 h before the administration of histamine hydrochloride.

Assay of Histamine H_2-Receptor Antagonistic Activity——*In Vivo* Anti-secretory Experiments. Anesthetized Rat Perfused Stomach Preparation: Rats were fasted for 24 h, then anesthetized with 1.25 g/kg urethane, and the perfused stomach preparation was obtained according to the modification by Sawada *et al.*[9] of the method of Ghosh and Schild.[10] A dual polyethylene cannula was introduced into the gastric lumen after ligation of the pylorus and esophagus. The stomach was continuously perfused with saline solution warmed at 30 °C through the gastric cannula at the rate of 1 ml/min. The 10-min perfusate was titrated with 1/100 N NaOH. Histamine·2HCl (Kishida Chemical Co., Ltd.) 1 mg/kg, tetragastrin (Peptide Institute Inc.) 10 μg/kg or carbachol (Tokyo Kasei Industry Co., Ltd.) 10 μg/kg was injected intravenously as a stimulant. Each sample was suspended in 0.2% CMC and administered intraduodenally.

Isolated Tissue Experiment. Guinea-pig Isolated Right Atrium: A piece of guinea-pig right atrium was mounted in a 20 ml bath containing Krebs solution at 32 °C and then histamine was added to the solution at a concentration of 1×10^{-7} to 1×10^{-3} mol/l. The spontaneous contraction frequency was measured to give cumulative response curves to histamine at 60-min intervals. After two constant control curves had been obtained, the sample was added to the bath and 45 min later a further response curve to histamine was obtained in the presence of the sample. Response curves were plotted as percent of maximum response against the log concentration of histamine.

Results

Inhibitory Activity of Each Fraction from 50% Methanol Extract of "Chang Zhu" on Gastric Secretion in Rats

As shown in Table I, the benzene fraction inhibited gastric secretion in pylorus-ligated rats but the methanol and aqueous fractions did not.

Inhibitory Activity of β-Eudesmol or Hinesol on Gastric Secretion in Rats

A shown in Tables II and III, β-eudesmol and hinesol significantly inhibited dose-dependently the amount of gastric juice, total acid output and total peptic activity after both oral and intraduodenal administration. Cimetidine and atropine, used as positive controls, also inhibited all these parameters.

Anti-ulcerogenic Activity in Rats

Effect on Pylorus-Ligated Ulcer——The effects of β-eudesmol and hinesol on ulceration in pylorus-ligated rats are shown in Table IV. β-Eudesmol, when administered orally or

TABLE I. Effect of Fractions from 50% Methanol Extract of *Atractylodes lancea* Rhizome on Gastric Secretion in Rats

Treatment (*p.o.*)	Dose (mg/kg)	No. of rats	Gastric volume (ml/100 g b.w.)
Control	—	10	4.33±0.29
Benzene fr.	200	10	2.17±0.19[a]
Methanol fr.	200	10	4.53±0.35
Aqueous fr.	200	10	4.34±0.45

a) Significantly different from the control group at $p<0.05$. All fractions were suspended in 0.2% CMC and were administered orally 1 h before pylorus-ligation.

TABLE II. Effects of β-Eudesmol and Hinesol on Gastric Secretion in Pylorus-Ligated Rats (4 h)

Treatment (*p.o.*)	Dose (mg/kg)	No. of rats	Gastric volume (ml/100 g b.w.)	Total acid output (μeq/100 g b.w.)	Total peptic activity (mg as tyrosine/100 g b.w.)
Control[a]	—	10	4.51±0.38	580.9±55.5	289.7±22.4
β-Eudesmol					
	2	10	2.99±0.25[b]	329.2±41.1[b]	170.0±21.3[b]
	10	10	2.77±0.27[c]	294.2±36.8[b]	151.6±18.7[b]
	50	10	1.64±0.16[d]	154.8±17.9[d]	92.6±8.9[c]
Hinesol					
	10	10	3.98±0.26	448.4±54.4	267.9±13.4
	50	10	3.32±0.28	398.4±34.4	242.6±29.9
	100	10	2.69±0.28[b]	333.3±35.0[b]	182.6±20.6[b]
Cimetidine	10	10	1.48±0.12[d]	99.7±13.3[d]	77.6±10.4[d]
Atropine sulfate	10	10	0.62±0.18[d]	43.7±17.1[d]	23.6± 7.4[d]

a) 0.2% CMC in water. All values represent the mean±S.E. All drugs were administered orally 1 h before pylorus ligation. Significantly different from the control group: *b*) $p<0.05$, *c*) $p<0.01$, *d*) $p<0.005$.

TABLE III. Effects of β-Eudesmol and Hinesol on Gastric Secretion in Pylorus-Ligated Rats (4 h)

Treatment (i.d.)	Dose (mg/kg)	No. of rats	Gastric volume (ml/100 g b.w.)	Total acid output (μeq/100 g b.w.)	Total peptic activity (mg as tyrosine/100 g b.w.)
Control[a]	—	10	3.78±0.38	393.5±45.3	230.7±20.3
β-Eudesmol					
	2	10	2.80±0.68	302.4±40.4	188.3±48.3
	10	10	2.76±0.35[b]	289.2±48.2	151.6±18.7[b]
	50	10	1.65±0.53[c]	188.5± 76.7[c]	82.6± 8.8[c]
Hinesol					
	10	10	3.34±0.44	325.5±44.5	221.1±32.5
	50	10	2.82±0.30	296.4±47.6	206.4±22.8
	100	10	2.74±0.44[b]	266.8±66.7[b]	193.7±20.4[b]
Cimetidine	10	10	2.09±0.65[b]	126.8±33.9[c]	106.9±38.0[c]
Atropine sulfate	10	10	0.89±0.19[d]	98.3±26.8[d]	22.8± 8.9[d]

a) 0.2% CMC in water. All values represent the mean±S.E. All drugs were administered intraduodenally immediately after pylorus ligation. Significantly different from the control group: *b*) $p<0.05$, *c*) $p<0.01$, *d*) $p<0.005$.

intraduodenally, prevented gastric ulceration as effectively as cimetidine (10 mg/kg) at the dose of 10 mg/kg. Hinesol also showed a preventive effect at the dose of 100 mg/kg. Atropine greatly decreased the ulcer index.

TABLE IV. Effect of β-Eudesmol and Hinesol on Gastric Ulceration in Pylorus-Ligated Rats

Treatment	Dose (mg/kg)	Route	No. of rats	Ulcer index (mean ± S.E.)	Route	No. of rats	Ulcer index (mean ± S.E.)
Control[a]	—	*p.o.*	10	3.50 ± 0.37	i.d.	10	3.89 ± 0.50
β-Eudesmol							
	2	*p.o.*	10	2.83 ± 0.22	i.d.	10	2.73 ± 0.28
	10	*p.o.*	10	1.50 ± 0.49[c]	i.d.	10	1.63 ± 0.50[c]
	50	*p.o.*	10	1.02 ± 0.23[c]	i.d.	10	1.05 ± 0.53[c]
Hinesol							
	10	*p.o.*	10	3.20 ± 0.33	i.d.	10	3.60 ± 0.38
	50	*p.o.*	10	3.12 ± 0.28	i.d.	10	3.08 ± 0.28
	100	*p.o.*	10	2.23 ± 0.39[b]	i.d.	10	2.03 ± 0.38[b]
Cimetidine	10	*p.o.*	10	1.48 ± 0.62[c]	i.d.	10	1.99 ± 0.67[b]
Atropine sulfate	10	*p.o.*	10	0.78 ± 0.23[d]	i.d.	10	0.88 ± 0.45[c]

a) 0.2% CMC in water. All drugs were administered orally 1 h before or intraduodenally immediately after pylorus ligation. Significantly different from the control group: *b*) $p<0.05$, *c*) $p<0.01$, *d*) $p<0.005$.

TABLE V. Effect of β-Eudesmol and Hinesol on Aspirin-Induced Ulceration in Pylorus-Ligated Rats

Treatment (*p.o.*)	Dose (mg/kg)	No. of rats	Ulcer index (mean ± S.E.)
Control[a]	—	10	35.25 ± 3.09
β-Eudesmol			
	10	10	27.63 ± 2.11
	50	10	18.55 ± 3.19[c]
Hinesol			
	50	10	34.71 ± 6.63
	100	10	22.75 ± 8.23[b]
Atropine sulfate	10	10	12.32 ± 2.38[c]

a) 0.2% CMC in water. Each drug was administered orally 1 h before the injection of aspirin. Significantly different from the control group: *b*) $p<0.05$, *c*) $p<0.01$.

TABLE VI. Effect of β-Eudesmol and Hinesol on Histamine-Induced Ulceration in Rats

Treatment (*p.o.*)	Dose (mg/kg)	No. of rats	Ulcer index (mean ± S.E.)
Control[a]	—	10	2.83 ± 0.32
β-Eudesmol			
	10	10	1.99 ± 0.38
	50	10	1.33 ± 0.68[b]
Hinesol			
	50	10	2.43 ± 0.65
	100	10	2.23 ± 0.44
Cimetidine	25	10	1.13 ± 0.33[b]

a) 0.2% CMC in water. Each drug was administered orally 1 h before the injection of histamine. Significantly different from the control group: *b*) $p<0.05$.

Aspirin-Induced Gastric Lesion——The preventive effects of β-eudesmol and hinesol on aspirin-induced ulcer were examined. As shown in Table V, β-eudesmol and hinesol decreased the ulcer index at the doses of 50 and 100 mg/kg, respectively. Atropine markedly decreased

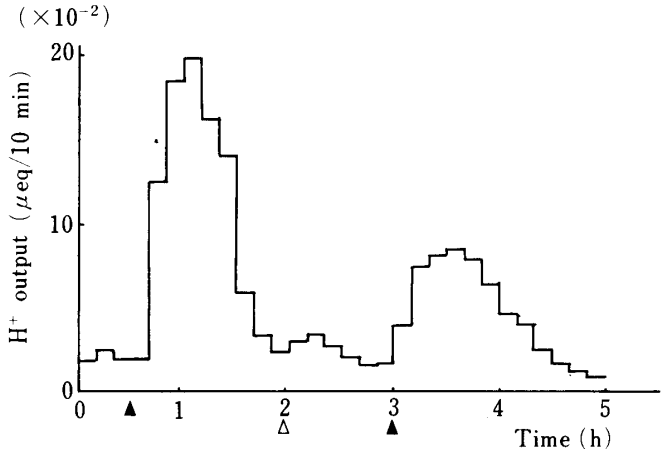

Fig. 1. Effect of β-Eudesmol on Gastric Secretion by Histamine in the Perfused Rat Stomach Preparation

Mean values of 8 rats per group. △, β-eudesmol 50 mg/kg i.d. ▲, histamine 1.0 mg/kg i.v.

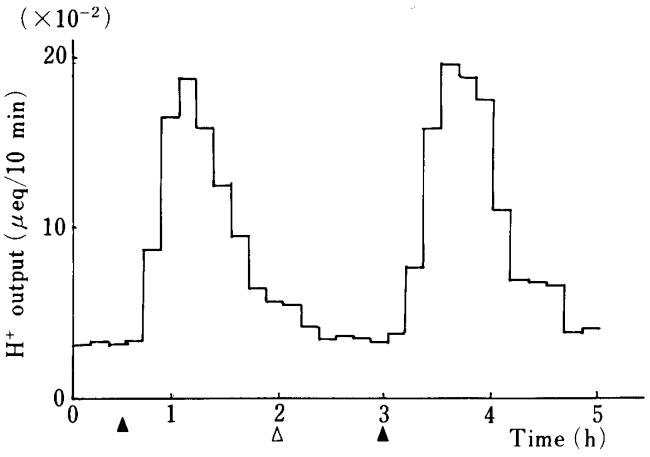

Fig. 2. Effect of Hinesol on Gastric Secretion by Histamine in the Perfused Rat Stomach Preparation

Mean values of 8 rats per group. △, hinesol 100 mg/kg i.d. ▲, histamine 1.0 mg/kg i.v.

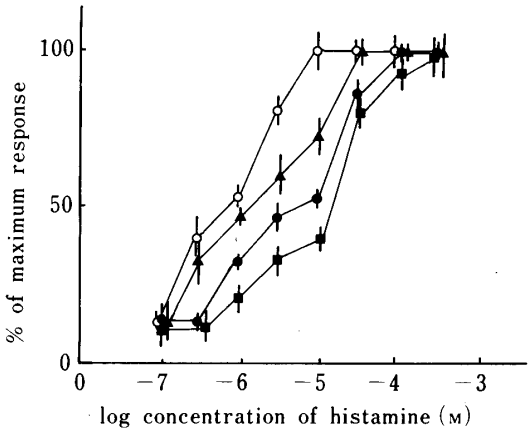

Fig. 3. Effect of β-Eudesmol on the Histamine-Induced Contraction Frequency of Guinea-pig Isolated Right Atria

Response to histamine alone (○) and in the presence of β-eudesmol 1×10^{-5} M (●), 1×10^{-6} M (▲), and cimetidine 1×10^{-6} M (■). Each point is the mean from at least 4 preparations; vertical lines show S.E.

the ulcer index at the dose of 10 mg/kg.

Histamine-Induced Gastric Lesion——The preventive effects of β-eudesmol and hinesol on histamine-induced gastric lesion were examined. As shown in Table VI, β-eudesmol markedly decreased the ulcer index dose-dependently at the doses of 50 and 10 mg/kg, but hinesol had no effect even at the dose of 100 mg/kg. Cimetidine decreased the ulcer index at the dose of 25 mg/kg.

H_2-Receptor Antagonistic Activity

***In Vivo* Anti-secretory Experiments on Anesthetized Rat Perfused Stomach Preparation**——The effects of β-eudesmol and hinesol on the gastric acid secretion stimulated by histamine in the perfused stomach preparations are shown in Figs. 1 and 2. Intraduodenal administration of β-eudesmol at the dose of 50 mg/kg markedly inhibited the gastric acid secretion but hinesol had no effect at the dose of 100 mg/kg.

β-Eudesmol had no inhibitory activity on the gastric acid secretion stimulated by tetragastrin or carbachol.

Isolated Tissue Experiments. Guinea-pig Isolated Right Atrium——The effects of β-eudesmol and cimetidine on the guinea-pig right atrium response to histamine are shown in

Fig. 3. Cimetidine at the concentration of 1×10^{-6} mol/l produced a shift to the right of the histamine concentration curve without depressing the maximum response, and β-eudesmol produced a similar dose-related shift to the right.

Discussion

The results show that the benzene extract, an essential oil fraction obtained from the 50% methanol extract of *Atractylodes lancea* rhizome, possesses significantly inhibitory activity on gastric acid secretion in pylorus-ligated rats. Two sesquiterpenes, β-eudesmol and hinesol, separated as major components from the benzene extract exhibited, after both oral and intraduodenal administration, greater anti-secretory activity than the benzene extract.

β-Eudesmol dose-dependently inhibited the ulcers in Shay rats, as well as histamine- and aspirin-induced gastric ulcers. Hinesol also showed inhibitory activity against Shay ulcers and aspirin-induced gastric ulcers but was inactive in inhibiting the induction of ulcers by histamine. Thus, these two components are considered to be the active principles of the anti-ulcerogenic activity of *Atractylodes lancea* rhizome.

β-Eudesmol showed a marked anti-secretory activity on gastric acid secretion stimulated by histamine in a perfused rat stomach preparation, but hinesol did not. β-Eudesmol competitively antagonized the action of histamine on the guinea-pig atrium, and its antagonistic activity was comparable to that of cimetidine. On the other hand, β-eudesmol did not show anti-secretory activity on tetragastrin- or carbachol-stimulated gastric acid secretion. Consequently, it seems that the inhibitory activity of β-eudesmol on gastric acid secretion can be attributed to its antagonistic effect on histamine H_2-receptor. The H_2-antagonistic effect of β-eudesmol should be confirmed in further studies.

Since hinesol did not exert competitive inhibition on histamine-induced response in rat stomach or guinea-pig atrium, it is suggested that H_2-receptor is not involved in the mechanism of anti-secretory action of hinesol.

Regarding the developmental mechanism of aspirin-induced ulcer, it is considered that the lesion of mucous membrane is caused by aspirin through the inhibition of mucus secretion as a result of blockage of mucosubstance biosynthesis in gastric mucosa, and the consequent erosion is promoted by back-diffusion of gastric acid at the lesion. Thus gastric acid secretion is an important factor for the development of aspirin-induced ulcer. Therefore the anti-secretory action of β-eudesmol and hinesol is probably responsible for the preventive effect against aspirin-induced ulcer. Okabe and Kawakami[11] reported that cimetidine was strongly active against the aspirin model, and its main mechanism of action appears to be inhibiting gastric acid secretion in addition to the cytoprotective action. Indeed, Guth *et al.*[12] also reported that cimetidine possesses cytoprotective activity analogous to that of prostaglandins.

It was concluded that the anti-ulcerogenic effect of 50% methanol extract from *Atractylodes lancea* rhizome can be primarily attributed to the anti-secretory action of β-eudesmol in addition to that of hinesol. The mechanism of the anti-secretory effect of β-eudesmol may be mainly due to histamine H_2-receptor blocking action. However, hinesol appears to inhibit the gastric secretion by some unknown mechanism. The strongly inhibitory effects of β-eudesmol and hinesol on aspirin-induced ulcer and on the other models of gastric ulceration might also be related to some protective factors such as increased production of mucus in the gastric mucosa. Further studies on the mode of anti-ulcerogenic action of β-eudesmol and hinesol using different approaches are necessary.

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