Effect of Extracts of Zingiberaceae Herbs on Gastric Secretion in Rabbits

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Some of the Zingiberaceae herbs are known to be useful as stomachics. Water extracts and methanol extracts of eight such herbs were examined in intact unanesthetized rabbits for their effect on gastric secretion. Oral administration of either water extracts or methanol extracts caused a significant decrease in gastric secretion. A significant effect of these extracts appeared at 3 h after administration. The effect of water extracts on gastric secretion was very similar to that of cimetidine, with a significant decrease in acid output. The effect of the methanol extracts was primarily observed as decreased pepsin output.

Keywords stomachic herb; extraction; rabbit; gastric secretion; acid output; pepsin output; oral administration

Herbs of Zingiberaceae have been widely used as aromatic stomachics since ancient times.[1-3] Many formulae are recorded in The Pharmacopeia of Japan (JPX).

Gastric irritation often appears in conjunction with an increase in acid output, an increase in peptic activity, and/or a decrease of mucoprotein content.[4] It has been reported that the effect of powdered gentian as a stomachic is due to a decrease in total grastric secretion.[5] Since herbal drugs of Zingiberaceae are believed to have antiulcer properties,[6] it is of interest to investigate the effect of these herbs on gastric secretion.

In the present study, we examined the effect of water extracts and methanol extracts of herbs of Zingiberaceae, in comparison with cimetidine, on gastric secretion in intact rabbits without pylorus ligation. Eight herbs of Zingiberaceae were tested. Water extracts and methanol extracts were used to compare the efficacy of either solvent in extracting effective component(s). We have previously reported the effect of anticholinergic agents and other synthetic drugs on gastric secretion of the intact unanesthetized rabbit.[7,8]

Although rats with pyloric ligation have frequently been used to screen for such drug activity, we chose to use intact rabbits in the present study in order to avoid stress from surgery such as incision of the abdomen and gastric distension. In our model, gastric juice is easily collected with an indwelling esophageal catheter.

Experimental

Materials Herbs used in the present study, listed in Table I, were obtained from Tochimoto Tenkaido Co. at the Osaka Market in 1982. They were finely chopped to a size of less than 2 mm. Extraction from each of the herbs was either in warm water (ten-fold excess by weight) or in methanol (ten-fold excess by weight). For water extraction, herbs were immersed in deionized water for 16 h at room temperature, and then warmed at 60 °C for 3 h. Each extract obtained was evaporated under reduced pressure at 40 °C to obtain the residue. For methanol extraction, herbs were immersed in methanol for 16 h at room temperature, and then warmed at 60 °C for 3 h. Each extract obtained was evaporated under reduced pressure at room temperature to obtain the residue. Five extractions were performed with samples of each of the herbs, and each individual extract was tested in a different rabbit. The extracts were kept in a desiccator before use. The codes for and yields of extracts of the eight herbs are listed in Table I. The dose given to rabbits was determined from the normal dose for humans, by calculating the dose per kg body weight according to references 1 to 3. Cimetidine was obtained commercially and dosed at 50 mg/kg. Other reagents used were of analytical grade.

Animal Study Male albino rabbits, weighing 3.0 to 3.5 kg, were obtained from Keari (Osaka), and were fasted for 24 h prior to experiments, but water was given freely. Further, whole gastric juice was aspirated before the experiments described below. For administration, water extracts were dissolved in 10 ml of distilled water; methanol extracts were suspended in 8 ml of distilled water after addition of lactose (5-fold excess over the dry weight of extract). Lactose was added as a thickener. The administration of each test solution was directly into the stomach *via* the indwelling gastric catheter, and then 2 ml of distilled water was administered to flush the catheter. Ten milliliters of distilled water or 8 ml of distilled water containing lactose was administered as an inactive control.

Collection of gastric juice was performed before and after administration of test solution (by the method reported previously[8]) as follows. Whole gastric juice was aspirated *via* the gastric catheter. Gastric juice as basal gastric secretion was collected for 15 min, from 45 min after an initial aspiration to remove the gastric content at the beginning of an experimental regimen. After drug administration, gastric juice was col-

TABLE I. Yields[a] of Extract from Eight Stomachic Herbs and the Dose[b] Administered to Rabbits

Herb (Place of origin)	Water extract		Methanol extract	
	Yield (% w/w)	Dose (mg/kg)	Yield (% w/w)	Dose (mg/kg)
Amomum cardamomum LINNAEUS (Cambodia)	17.7±1.2	111.9± 8.1	8.6±0.6	53.6±5.5
Curcuma longa LINNAEUS (Guangdong, China)	13.2±0.6	132.0± 6.2	15.5±0.9	155.0±8.5
Curcuma zedoaria ROSCOE (Guangxi, China)	8.2±0.9	136.9±14.3	8.2±0.5	136.9±8.9
Amomum xanthioides WALLICH (Thailand)	13.6±0.6	91.1± 4.4	7.2±0.6	48.2±4.1
Zingiber officinale ROSCOE (Yunnann, China)	16.9±0.5	169.0± 4.6	11.4±0.3	114.0±3.5
Elettaria cardamomum MATON (Malabar Coast, India)	12.6±1.2	126.9±12.3	10.9±0.8	109.0±8.0
Alpinia oxyphylla MIQUEL (Guangdong, China)	14.1±0.9	141.0± 9.4	8.5±0.6	85.0±6.1
Alpinia officinarum HANCE (Guangdong, China)	15.1±0.7	75.5± 0.4	14.1±1.0	70.5±5.0

a) Each value represents the mean ± S.E. ($n=5$); yield is given as percent of extract with respect to the weight of herbs used for extraction. *b*) Dose was determined from the dose of each herb in references 1 to 3, as described in the experimental section.

lected at 1 h intervals for 6 h. Each collection of gastric juice was performed during the final 15 min of each 1-h interval. This collection interval was based on the previous report,[7] in which rabbits treated similarly retained less than 5% of phenol red in the stomach at 45 min after administration. Gastric juice volume, pepsin output and total acid output were determined for each sample. Since gastric juice obtained was colorless, no bleeding and no back-flow of intestinal juice are considered to have occurred.

Assay Procedures Assays of pepsin activity[9] and total acid activity[10] in gastric juice were performed by common clinical methods.

Analyses of Data Changes in gastric secretion after administration of extracts were calculated as percent change with respect to the basal value of gastric secretion before the administration.

Statistical Analyses Statistical analyses were performed by the use of Student's *t* test.

Results and Discussion

Basal gastric secretion in rabbits before drug administration is shown in Table II. Administration of water or lactose solution caused no change in gastric secretion (Table II). Despite the frequent collection of gastric juice, the volume and constituents in gastric juice at each collection were not significantly different during the experimental period. These observations indicate that gastric secretion is not directly affected by our sampling technique. However, gastric secretion observed in the present study is considered to be an apparent gastric secretion, because much of the total output is lost through the pylorus in the 45 min interval between samples.

An oral administration of cimetidine at a dose of 50 mg/kg resulted in a decrease in pepsin and acid output and also gastric juice volume (Fig. 1). The effect of cimetidine appeared rapidly with decreased gastric secretion becoming constant after 2 h (Fig. 1). The decrease in acid output was more pronounced than other parameters measured, and was reflected as a higher pH value.

It has been reported[11] that cimetidine at a dose of more than 30 mg/kg in pylorus-ligated rats resulted in a significant decrease in acid output and further that cimetidine at a dose of 100 mg/kg induced decreases in both gastric juice volume and pepsin output; *i.e.*, an anticholinergic effect of cimetidine appears at a high dose. In the present study, it is not clear whether the decrease in gastric juice volume by cimetidine occurs through a suppressive secretion or through an acceleration of gastric emptying. Thus, the results obtained in the present study refer to 'apparent gastric secretion.' In a previous report,[7] anticholinergic agents decreased gastric juice volume by about 30 to 40% in this animal model, an effect similar to that observed with cimetidine in the present study.

To evaluate the effect of extracts of herbs on gastric secretion, changes in acid output and pepsin output were compared with changes in gastric juice volume. We believe that cimetidine caused a significant decrease in acid output in rabbits, because the decrease in acid output was greater

TABLE II. Basal Gastric Secretion and Gastric Secretion after Administration of Water Either with or without Lactose as a Control

Extract Code No.	Gastric secretions		
	Gastric volume (ml/h)	Total acid output (μeq/h)	Pepsin output (μeq/h)
(1) Group A: in the rabbits for study of water extracts			
Basal gastric secretion: before administration	9.6±0.3	1109±45	13.8±0.4
Control: after administration of water	9.5±0.2	1087±45	13.2±0.3
(2) Group B: in the rabbits for study of methanol extracts			
Basal gastric secretion: before administration	9.6±0.2	1060±39	13.7±0.3
Control: after administration of lactose solution	9.3±0.4	1036±16	13.5±0.3

Note: In the present study, 5 rabbits were used for each group of A and B. In group A, rabbits were subjected to a cross-over study of water alone and water extracts of eight herbs. In group B, rabbits were subjected to a cross-over study of lactose solution alone and methanol extracts of eight herbs. Thus, 9 experiments were performed with each rabbit. Since basal gastric secretion was determined in each experiment, numbers of data for basal gastric secretion were 45 for each group. No significant change in gastric secretion was observed in a control study after administration of either water alone or lactose solution alone. Six collections for determination of gastric juice secretion of each rabbit were used. Thus, numbers of data for the control were 30 for each group. Each value represents the mean±S.E. ($n=45$ for basal gastric secretion and $n=30$ for control).

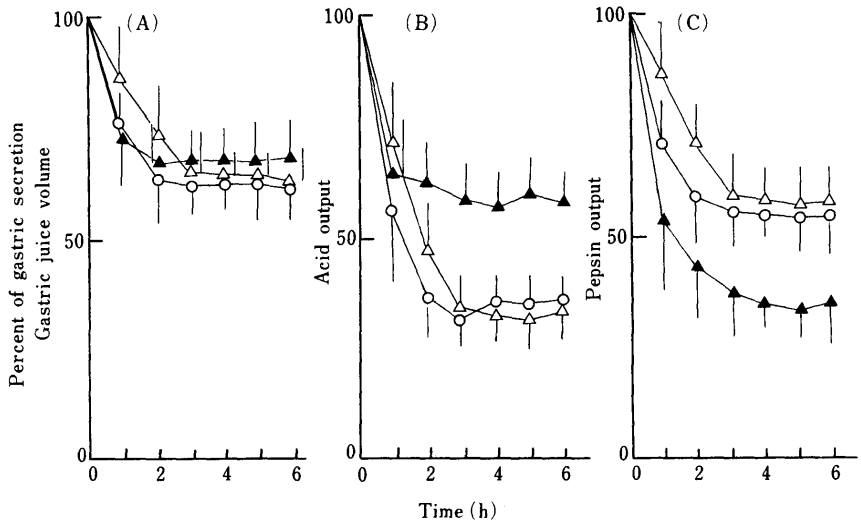

Fig. 1. Profiles of Gastric Secretion (A, Gastric Juice Volume; B, Acid Output; C, Pepsin Output) in Rabbits after Administration of Cimetidine (50 mg/kg, ○), or Water Extract (△) or Methanol Extract of *Zingiber officinale* ROSCOE (▲)

Each value represents the mean±S.E. ($n=5$).

TABLE III. Effect of Extracts of Eight Herbs on Gastric Secretion[a] in Rabbits after Oral Administration

Extract Herb (Code No.)	Gastric secretion (Percent of the basal secretion)		
	Gastric volume ml/h (%[a])	Acid output μeq/h (%[a])	Pepsin output μeq/h (%[a])
Water extracts (rabbits in group A)			
None	9.5±0.2 (98.7)	1020± 35 (92.1)	12.6±0.4 (91.3)
(1)	6.1±0.8[c] (63.2)	544± 83[c] (49.1)	7.7±1.2[c] (56.1)
(2)	5.3±0.8[c] (55.6)	371± 45[c] (33.5)	6.8±1.2[c] (49.6)
(3)	7.6±0.7[c] (79.5)	640± 75[c] (57.7)	9.4±0.7[c] (67.8)
(4)	6.8±0.6[c] (70.6)	465± 58[c] (41.9)	9.4±1.0[c] (67.8)
(5)	6.1±0.4[c] (63.7)	374± 50[c] (33.7)	7.6±1.2[c] (55.4)
(6)	6.9±0.8[c] (72.3)	472± 53[c] (42.6)	8.1±1.1[c] (59.1)
(7)	6.9±0.4[c] (72.0)	567± 45[c] (51.1)	9.2±0.4[c] (66.9)
(8)	6.5±0.2[c] (67.6)	491± 26[c] (44.3)	9.3±0.2[c] (67.2)
Methanol extracts (rabbits in group B)			
None	9.3±0.4 (97.2)	1034± 16 (97.6)	13.5±0.4 (98.2)
(1)	5.7±0.2[c] (59.3)	558± 51[c] (52.7)	6.0±0.4[c] (43.5)
(2)	5.0±0.7[c] (52.1)	474±100[c] (44.7)	4.3±0.6[c] (31.4)
(3)	6.4±0.9[c] (66.5)	576±104[c] (54.3)	5.6±1.4[c] (40.9)
(4)	6.0±0.5[c] (62.2)	624± 43[c] (58.9)	5.6±0.9[c] (40.8)
(5)	6.4±0.5[c] (66.4)	581± 42[c] (54.8)	4.5±0.7[c] (33.0)
(6)	5.2±0.4[c] (54.6)	532± 41[c] (50.2)	5.3±0.6[c] (38.6)
(7)	6.8±0.8[c] (70.7)	664± 49[c] (62.7)	7.8±0.9[c] (57.2)
(8)	7.2±0.3[c] (59.6)	630± 32[c] (59.4)	4.7±0.2[c] (34.2)
Cimetidine[b]			
	5.8±0.6[c] (60.1)	372± 52[c] (33.6)	7.6±0.5[c] (55.4)

a) Change in gastric secretion from 3 to 5 h after an administration of extracts was determined as percent of the basal value in Table II. *b*) The effect of cimetidine was examined at a dose of 50 mg/kg. Each value represents the mean±S.E. ($n=15$, 5 rabbits and three collections for each rabbit). *c*) $p<0.05$ *versus* none.

Water extracts and methanol extracts were obtained from the following herbs: (1) *Amomum cardamomum* LINNAEUS; (2) *Curcuma longa* LINNAEUS; (3) *Curcuma zedoaria* ROSCOE; (4) *Amomum xanthioides* WALLICH; (5) *Zingiber officinale* ROSCOE; (6) *Elettaria cardamomum* MATON; (7) *Alpinia oxyphylla* MIQUEL; (8) *Alpinia officinarum* HANCE.

than the decreases in either gastric volume or pepsin output.

An oral administration of either the water extract or the methanol extract of any of the eight herbs resulted in a decrease in the gastric secretion (Fig. 1 and Table III). As an example, the significant decrease in the gastric secretion (gastric juice volume, acid output and pepsin output) 3 h after administration of either water extract or methanol extract of *Zingiber officinale* ROSCOE is shown in Fig. 1. Similar results were obtained with the extracts of all herbs examined. In the present study, thus, the results after administration of the extracts and cimetidine were compared (mean values of three collections at 3, 4 and 5 h after the administration). These data are summarized in Table III.

After administration of water extract a significant decrease in acid output was observed, as with cimetidine; the decrease was greater than that in gastric juice volume except for extracts of *Amomum cardamomum* LINNAEUS and *Curcuma zedoaria* ROSCOE. The extents of decrease in both gastric juice volume and pepsin output after administration of all the water extracts were quite similar to those after administration of cimetidine (Table III).

The methanol extracts produced a significant decrease in pepsin output, which was greater than the decrease in gastric juice volume except for the extract of *Alpiniae oxyphylla* MIQUEL. However, the decrease in acid output caused by the methanol extracts could be accounted for by the decrease in gastric juice volume.

Recently, we found that administration of an extract of *Alpinia officinarum* HANCE to pylorus-ligated rats caused a significant dose-dependent decrease in gastric juice volume, *i.e.*, the extract showed an anticholinergic effect in pylorus-ligated rats (unpublished data). It is difficult to compare results from intact rabbits and pylorus-ligated rats. However, we believe that the decrease in gastric juice volume after administration of either extract in the rabbit model is due to a decrease in secretion.

It is considered that a common cause of gastric ulcer is high acid output.[12] For this type of ulcer, water extracts of Zingiberaceae should be rational therapy. It has been reported[13] that a decrease in pepsin output reduced gastric irritation. Thus, the administration of the methanol extract of these herbs showed be a more effective treatment for pepsin-induced gastric ulcer. Data presented herein suggest a qualitative difference in activity between water and methanol extracts. Further, it is considered that the active component(s) in the water extract and in the methanol extract are generally present in plants of the family Zingiberaceae.

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