Anti-ulcer Effects of Trichosanthes Fruits

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Anti-ulcer activities of fruits of *Trichosanthes kirilowii* MAXIMOWICZ var. *japonica* KITAMURA (50% ethanolic extract, TKE) were investigated in rats.

TKE, at doses of 100—1000 mg/kg, showed potent protection against experimental gastric lesions, namely, those induced by water-immersion, histamine, serotonin, HCl·ethanol, 0.6 N HCl, 0.2 N NaOH, 35% NaCl, and Shay' ulcer and acetic acid-induced gastric ulcer.

At doses of 500—1000 mg/kg, TKE decreased the gastric secretion and acid output in pylorus-ligation (for 7 h), but 100 mg/kg of TKE had no influence on the gastric secretion. On the other hand, TKE exerted inhibition on the contractile responses of the isolated ileum of mouse to acetylcholine.

These results suggested that TKE has an anti-ulcer effect.

Keywords *Trichosanthes Kirilowii* var. *japonica*; fruit; 50% ethanolic extract; rat; anti-ulcer effect; gastric secretion; mouse; ileum; acetylcholine

In our previous paper,[1] we reported the aggravation of Shay's ulcer when the extract of Allii Bakeri Bulbus (bulb of *Allium bakeri*) was given to rats. Allii Bakeri Bulbus is frequently found together with Trichosanthis Fructus (fruit of *Trichosanthes kirilowii*, *T. kirilowii* var. *japonica*, *T. bracteata*, *etc.*) in Chinese medicinal prescriptions. Few pharmacological activities, depression of water-immersion stress induced gastric lesions of rats (50% methanol extract)[2] and a slight influence on human blood platelet aggregation (ethanol extract)[3] are found in Trichosanthes seeds. Accordingly, in this paper we report the effects of fruits of *Trichosanthes kirilowii* MAXIMOWICZ var. *japonica* KITAMURA on acute and chronic gastric lesions.

Experimental

Animals Male Wistar rats weighing between 160—210 g and ddy mice weighing 20—27 g were used. They were housed in cages with mesh bottoms to prevent coprophagia and kept at 23±1 °C. The animals were starved for 24 or 48 h before use but allowed free access to water till the beginning of the experiment.

Materials Trichosanthes fruits (from Miyazaki prefecture, Japan) were purchased from Nakaikohshindo (Kobe). They (100 g) were extracted three times with 50% ethanol (1 l) at 90 °C for 2 h. Removal of the solvent *in vacuo* and drying of the extract produced a dark-brownish powder (TKE, 32.8 g). TKE was dissolved in bidistilled water and a constant injection volume of 0.5 ml/200 g was used for the rats; the vehicle alone was administered to the control group in the same manner.

Gastric Secretion in Pylorus-Ligated Rats Rats were killed by an overdose of ether after 7 h pylorus ligation. The gastric juice was collected and the volume was measured. Acid concentration was estimated by titration of the gastric juice with 0.05 N NaOH using a pH meter to endpoint pH 7.0. TKE was intraduodenally administered immediately after pylorus-ligation.

Water Immersion Stress-Induced Gastric Lesions According to the method of Takagi *et al.*,[4,5] rats were immobilized in a stress-cage and immersed vertically in a water bath at 23±1 °C to the level of the xiphoid process for 7 h. TKE was given orally immediately before stress treatment. The rats were killed by a blow on the head 7 h later. Subsequently, their stomachs were removed and inflated by injecting 10 ml of 2% buffered formalin. Each preparation was then incised along the greater curvature and examined for the presence of mucosal lesions. The length (mm) of each lesions was measured under a microscope (×7), summed per stomach, and used as the lesion index.

Pylorus-Ligated Rats (Shay Ulcer) The pyloric ligation was carried out according to the manner of Shay *et al.*[6] TKE was orally given immediately after pylorus ligation. Animals were killed 18 h later by an overdose of ether. Their stomachs were removed, inflated with 2% buffered formalin, incised along the greater curvature and examined for lesions developed in the forestomach. The longitudinal and abscissal length of each lesion was measured and the multiplied products were summed per stomach and used for an ulcer index. The index was established as follows: ulcer area 0 mm², 0; 1—6 mm², 1; 7—12 mm², 2; 13—18 mm², 3; 19—24 mm², 4; >24 mm² or perforation, 5.

Histamine-Induced Gastric Mucosal Lesions This model was carried out according to Büchner *et al.*,[7] and rats were administered histamine hydrochloride (Nacalai tesque) intraperitoneally at a dose of 300 mg/kg. TKE was given orally 30 min before administration of histamine. The rats were killed by a blow on the head 4 h later, and the stomachs were removed and inflated by injecting 10 ml of 2% buffered formalin. Each preparation was then incised along the greater curvature and examined for the presence of mucosal lesions. The length (mm) of each lesion was measured under a microscope (×7), summed per stomach, and used as the lesion index.

Serotonin-Induced Gastric Mucosal Lesions Rats were administered serotonin–creatinine sulfate (Merck) subcutaneously at 25 mg/kg dose. TKE was given orally 30 min before administration of serotonin. The rats were killed by a blow on the head 18 h later and the lesion index was obtained in the manner described above.

HCl·Ethanol-Induced Gastric Mucosal Lesions This model was carried out according to Mizui and Doteuchi,[8] and rats were given 1 ml of HCl·ethanol (60% ethanol in 150 mM HCl) orally. TKE was given orally 30 min beforehand. The rats were killed by a blow on the head 1 h later and the lesion index was prepared by the procedure mentioned above. In a separate experiment, hand, indomethacin (Nacalai tesque) suspended in saline with a trace of Tween 80 (Nacalai tesque) was given subcutaneously at a dose of 5 mg/kg 1 h before HCl·ethanol was administered.

Gastric Lesions Produced by Some Necrotizing Agents According to Robert *et al.*,[9] rats were orally given 1 ml of 0.6 N HCl, 0.2 N NaOH and 35% NaCl, respectively. TKE was given orally 30 min before receiving the necrotizing agents. The rats were killed by a blow on the head 1 h later and the lesion index gained by the process described above.

Acetic Acid-Induced Gastric Ulcer This ulcer model was carried out according to the method of Takagi *et al.*[10] Rats were anesthetized with ether and subjected to laparotomy to expose the stomach, after which 20 μl of 20% acetic acid was injected carefully under the subserosal layer of the abdominal side in the grandular stomach, then the abdomen was closed. Animals were fed normally after surgical operations. TKE was orally given (0.5 ml/200 g) once a day for 15 d from the third day after the operation. Rats were killed by a blow on the head and their stomachs were removed, inflated with 2% buffered formalin, and then incised along the greater curvature. The longitudinal and abscissal length of the ulcer·areas were measured and the multiplied product was used as the ulcer index.

Effect on Isolated Ileum Preparation of Mouse Ileum preparation isolated from mouse was suspended in a 50 ml organ bath filled with Tyrode's solution (temperature, 26 °C). TKE dissolved in Tyrode's solution was applied to the bath. The behavior of the ileum was mediated by transducer (Natsume KN-259) and recorded on a kimorecorder. Acetylcholine chloride (Nacalai tesque) was used as a spasmogenic.

Statistics Data are presented as the mean±S.E. Statistical analysis was performed using the Student's *t*-test. Values of $p<0.05$ were regarded as significant.

Results

Gastric Secretion in Pylorus-Ligated Rats The volume of gastric secretion and acid concentration after 7 h of pylorus-ligation are shown in Table I. Intraduodenal administration of TKE significantly (500 and 1000 mg/kg) decreased the gastric acid secretion dose-dependently. A dose of 1000 or 500 mg/kg also inhibited acid concentration, however, 100 mg/kg of TKE tended to increase it.

Water-Immersion Stress-Induced Gastric Lesions As shown in Table II, TKE at doses of 500 and 1000 mg/kg given by peroral administration revealed significant inhibition of the gastric lesions.

Pylorus-Ligated Rats Effect on pyloric ligation-induced gastric ulcer is exhibited in Table III. TKE significantly inhibited the development of the ulcer at doses of 500 and 1000 mg/kg given perorally, respectively.

Histamine-Induced Gastric Mucosal Lesions Histamine at 300 mg/kg administered intraperitoneally caused about 32.7 mm lesions in the glandular stomach in the control group and, as displayed in Table IV, TKE decreased the lesion index by 7.6, 29.9 and 43.2% at respective doses of 100, 500 and 1000 mg/kg; however, there was no significant difference.

Serotonin-Induced Gastric Mucosal Lesions Serotonin 25 mg/kg subcutaneous administration produced many streaky and speckled lesions in the glandular stomachs of rats. About 63 mm lesions per animal were observed in the control group. TKE significantly protected the rats from developing lesions by 59.1 and 63.6% at doses of 500 and 1000 mg/kg (Table V).

HCl·Ethanol-Induced Gastric Mucosal Lesions In all the control group rats given 1 ml of HCl·ethanol by peroral

Table I. Effects of TKE on Gastric Acid Secretion in Pylorus-Ligated Rats

Substance	Dose (mg/kg, i.d.)	No. of rats	Volume (ml)	Acidity (mEq/l)	Acid output (μEq/h)
Control		9	4.3±0.3	123.1±13.1	74.9± 8.1
TKE	100	9	4.3±0.6	158.9±18.2	103.6±21.9
	500	9	2.3±0.2[a]	120.4±24.4	37.9± 7.6[a]
	1000	9	0.5±0.1[a]	95.6±15.6	6.2± 1.1[a]

All values represent the mean±S.E. *a*) Significantly different from control, $p<0.01$.

Table II. Effect of TKE on Water-Immersion Stress-Induced Gastric Lesions in Rats

Substance	Dose (mg/kg, *p.o.*)	No. of rats	Lesion index (mm)	Inhibition (%)
Control		11	32.5±6.1	—
TKE	100	10	27.1±2.1	16.5
	500	10	15.9±2.1[a]	51.0
	1000	11	10.7±3.0[b]	66.9

All values represent the mean±S.E. Significantly different from control, *a*) $p<0.05$, *b*) $p<0.01$.

Table III. Effect of TKE on Gastric Ulceration in Pylorus-Ligated Rats

Substance	Dose (mg/kg, *p.o.*)	No. of rats	Ulcer index	Inhibition (%)
Control		11	3.8±0.5	—
TKE	100	10	2.1±0.7	44.4
	500	10	1.2±0.7[a]	68.2
	1000	10	0.6±0.3[a]	84.1

All values represent the mean±S.E. *a*) Significantly different from control, $p<0.01$.

Table IV. Effect of TKE on Histamine-Induced Gastric Lesions in Rats

Substance	Dose (mg/kg, *p.o.*)	No. of rats	Lesion index (mm)	Inhibition (%)
Control		10	32.7±6.8	—
TKE	100	10	30.2±4.9	7.6
	500	10	22.9±4.1	29.9
	1000	9	18.6±1.8	43.2

All values represent the mean±S.E.

Table V. Effect of TKE on Serotonin-Induced Gastric Lesions in Rats

Substance	Dose (mg/kg, *p.o.*)	No. of rats	Lesion index (mm)	Inhibition (%)
Control		10	63.3±6.8	—
TKE	100	10	51.8±8.0	18.1
	500	10	25.9±5.9[a]	59.1
	1000	10	23.1±6.9[a]	63.6

All values represent the mean±S.E. *a*) Significantly different from control, $p<0.01$.

Table VI. Effect of TKE on HCl·Ethanol-Induced Gastric Lesions in Rats

Substance	Dose (mg/kg, *p.o.*)	No. of rats	Lesion index (mm)	Inhibition (%)
Control		16	87.7±7.9	—
TKE	100	10	62.3±9.7	28.9
	500	10	17.1±3.5[a]	80.9
	1000	15	4.7±1.5[a]	94.8

All values represent the mean±S.E. *a*) Significantly different from control, $p<0.01$.

Table VII. Effect of TKE on HCl·Ethanol-Induced Gastric Lesions in Rats Pretreated with Indomethacin

Substance	Dose (mg/kg, *p.o.*)	No. of rats	Lesion index (mm)	Inhibition (%)
Control		10	119.0±15.2	—
TKE	100	10	93.6±14.6	21.4
	500	10	29.4±13.3[a]	75.3
	1000	10	14.0±12.7[a]	88.2

All values represent the mean±S.E. *a*) Significantly different from control, $p<0.01$.

Table VIII. Effect of TKE on 0.6 N HCl-Induced Gastric Lesions in Rats

Substance	Dose (mg/kg, *p.o.*)	No. of rats	Lesion index (mm)	Inhibition (%)
Control		18	49.4±5.9	—
TKE	100	11	20.2±4.2[a]	59.1
	500	10	3.7±1.6[a]	92.5
	1000	10	3.3±1.8[a]	93.4

All values represent the mean±S.E. *a*) Significantly different from control, $p<0.01$.

Table IX. Effect of TKE on 0.2 N NaOH-Induced Gastric Lesions in Rats

Substance	Dose (mg/kg, *p.o.*)	No. of rats	Lesion index (mm)	Inhibition (%)
Control		14	80.5±6.3	—
TKE	100	9	58.5±6.2[a]	27.3
	500	10	6.9±2.2[b]	91.4
	1000	15	6.4±4.0[b]	92.1

All values represent the mean±S.E. Significantly different from control, *a*) $p<0.05$, *b*) $p<0.01$.

Table X. Effect of TKE on 35% NaCl-Induced Gastric Lesions in Rats

Substance	Dose (mg/kg, *p.o.*)	No. of rats	Lesion index (mm)	Inhibition (%)
Control		10	93.8±10.3	—
TKE	100	10	84.7± 6.8	9.7
	500	9	65.2± 6.5[a]	30.5
	1000	9	65.5± 4.0[a]	30.2

All values represent the mean±S.E. *a*) Significantly different from control, $p<0.05$.

Table XI. Effect of TKE on the Healing Process of Acetic Acid-Induced Ulcer in Rats

Substance	Dose (mg/kg, *p.o.*)	No. of rats	Lesion index (mm)	Inhibition (%)
Control		10	10.1±2.5	—
TKE	100	7	6.8±2.3	32.8
	500	7	4.0±0.4	60.0
	1000	7	4.4±1.2	56.1

All values represent the mean±S.E.

administration, severe streaky mucosal lesions developed in the glandular stomach 1 h later. The mean length of the lesions was about 87 mm. Pretreatment with TKE at 30 min before HCl·ethanol administration significantly and dose-dependently inhibited these lesions (Table VI). On the other hand, pretreatment with 5 mg/kg indomethacin by subcutaneous administration at 1 h before HCl·ethanol was given accelerated development of the lesions. The protective effect of TKE was little less than the effect without indomethacin pretreatment at doses of 500 and 1000 mg/kg, but 100 mg/kg TKE by peroral administration resulted in no significant inhibition (Table VII).

Gastric Lesions Produced by Some Necrotizing Agents Administration of 1 ml of 0.6 N HCl, 0.2 N NaOH or 35% NaCl produced severe mucosal damage of the glandular stomach, as shown in Tables VIII, IX and X. Pretreatment with TKE significantly affected the extent of these necrotizing agents-induced gastric lesions. However, at doses of 100 mg/kg TKE did not show significant protection in 35% NaCl-induced gastric lesions.

Acetic Acid-Induced Gastric Ulcer As revealed in Table XI, TKE given at doses of 100, 500 and 1000 mg/kg daily for 15 d showed a healing effect on acetic acid-induced ulcers, though no significant difference from the control value was found.

Effect on Isolated Ileum Preparation of Mouse As illustrated in Fig. 1, TKE at doses between 2.5×10^{-3} and

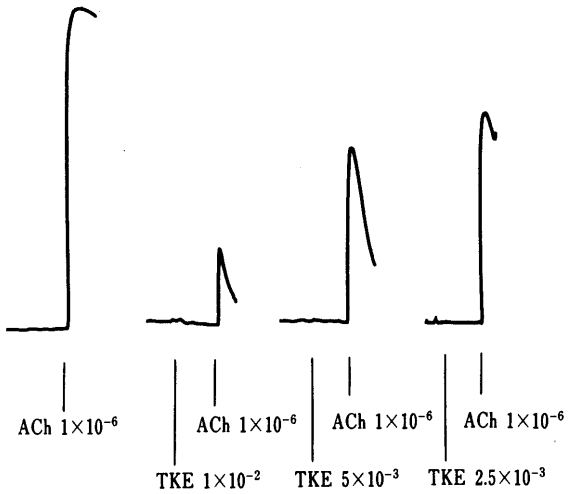

Fig. 1. Effect of TKE on the Contraction of Mouse Ileum Induced by Acetylcholine

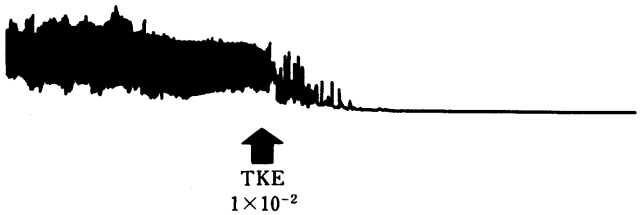

Fig. 2. Effect of TKE on Spontaneous Motility of Mouse Ileum

1.0×10^{-2} g/ml was found to have an effective relaxing action on the acetylcholine(1×10^{-6} g/ml)-induced contraction of the ileum in mouse. Further, 1×10^{-2} g/ml of TKE suppressed the spontaneous movement of the ileum in mouse (Fig. 2).

Discussion

It was suggested that TKE inhibits gastric acid secretion and has an anti-ulcer effect at higher doses. The former action appears when TKE is intraduodenally given, and thus seems to occur after its absorption through the intestinal tract by some mechanism.

Although it is less than the cases of Trichosanthis Semen (seed of *Trichosanthes kirilowii*, 2000 mg/kg of 50% methanol extract, in rat, 89.9% prevention)[2] and Trichosanthis Radix (1090 mg/kg of hot water extract, in mouse, 77.0% prevention),[11] TKE (1000 mg/kg, in rat, 66.9% prevention) prevented water-immersion stress-induced gastric lesions when orally applied 30 min beforehand. The acute gastric mucosal necrotizing caused by stress is generated in the course of the disturbance of gastric mucosal microvascular circulation, resulting in an increase of mucosal fragility and strain of the autonomic nervous system[12,13]; muscarinic cholinergic neuron[14] plays especially an important role in this case. In addition, the acceleration of gastric acid secretion is also presumed to be one of the causes of the necrotizing.[13] TKE decreases the gastric acid secretion at doses of 500 and 1000 mg/kg and depresses the effect of acetylcholine on isolated intestine of mouse dose-dependently. These results suggest that the preventive effect of TKE against stress-induced gastric lesion is due to its inhibition of gastric acid secretion based on atropine-type

anti-cholinergic action.

Shay ulcer is caused by peptic activity due to remaining gastric juice, and an antacid or spasmolysant remarkably inhibits this ulcer.[15] As TKE was orally given immediate after pylorus ligation, we could observe the direct effect on the gastric mucosa to be presumed on the significant inhibition of Shay ulcer formation at the rate of 84.1% by a 1000 mg/kg dosage.

The effect of TKE upon the gastric mucosal damage induced by serotonin was considerably more than that induced by histamine.

It was found that TKE exhibits preventive effects on HCl·ethanol-, 0.6 N HCl-, 0.2 N NaOH- and 35% NaCl-induced gastric lesions in rats. Especially, gastric lesions induced by ethanol are a typical method for assessing the occurrence of "cytoprotection" in a drug, and it is presumed that direct cell necrosis,[16] acceleration of gastric movement[17] and so on contribute to the appearance of a lesion irregardless of the presence of acid. Further, as HCl exists in the stomach, it is considered that depression of acid secretion is not the cause of anti-lesion.[18] TKE suppressed this HCl·ethanol-induced gastric lesion even at 100 mg/kg dose, which had little influence on gastric juice secretion. Moreover, TKE significantly repressed the gastric lesions resulting from 0.6 N HCl or 0.2 N NaOH at the same dosage, 100 mg/kg. Taking these matters into consideration, "cytoprotection" was capable of being one of the anti-ulcer effects of TKE. Although "cytoprotection" of some drugs is occasionally mediated by endogenous prostaglandins,[19,20] it is difficult to regard these prostaglandins as also contributing to the effect of TKE, because a HCl·ethanol-induced gastric lesion pretreated with indomethacin (at 5 mg/kg, no damageable dose to stomach within 2 h[21]) was similarly prevented by TKE.

Since TKE tended to depress the acetic acid-induced ulcer, it is also expected to have a healing effect on a chronic ulcer.

It was shown that TKE exhibits a protective effect on acute and chronic gastric lesions in rats, however, details of its mechanisms are not yet well defined. The participation of "cytoprotection" and inhibition of gastric acid secretion through anti-cholinergic activity is presumed.

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