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Prevention Action of Turmeric against HCl-induced Gastric Necrosis in Rat : to Verify the Mode of Action of Previous Clinical Study

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ABSTRACT: The purposes of this scientific work were to study the mode of action of turmeric in animal and to verify previous clinical data of turmeric action in peptic ulcer patient. Oral administration of 0.6 N HCl to fasted rats produced extensive necrosis of gastric mucosa. Pretreatment with crude turmeric suspension (pH 5.5) orally prevented the necrosis while pretreatment with suspension of ground straw fiber from rice leaves showed no protection. This property of turmeric represented "cytoprotective" action. Although the mechanism of gastric cytoprotection was unknown whether it acted physically, biochemically, or pharmacologically, turmeric appeared to increase the resistance of gastric mucosal cells to the necrotizing effect of strong irritant such as 0.6 N HCl used. These results suggest that turmeric, by some mechanisms which need furtherance proof, maintains the cellular integrity of gastric mucosa and might be beneficial for the treatment of a variety of diseases in which gastric mucosal injury is present..

KEY WORDS: Turmeric, peptic ulcer, gastric necrosis, cytoprotective action

INTRODUCTION

Turmeric, *Curcuma longa* Linn. or *Curcuma domestica* Val., is a medicinal plant of Southeast Asia. This plant has been used to treat abdominal pain which sounds like the pain caused by peptic ulcer (1). It has been used in empirical medicine for centuries by Thai traditional healers (2). A group of researchers has tried to prove this property scientifically through endoscopic findings in human subjects (3, 4). The result showed that only 50% of cases was healed while there was an evidence that placebo had 40% of healing effect (5). The explanation for this low therapeutic result was either crude turmeric preparation had mild action or it had no real action on peptic ulcer. It is intriguing, therefore, to verify either of these actions through animal experiments. Hopefully, animal experiments can provide good results, i.e., crude turmeric can prevent HCl-induced ulcer. This will be the answer that crude turmeric is beneficial to the peptic ulcer patients despite it has mild action. Hence, crude turmeric powder can be used as a remedy for peptic ulcer according to the reports form a group of researchers (3, 4).

The data from animal study here will support the previous research in human. It will form scientific information and confirm clinical application of turmeric.

METHODS

In all the studies reported here, 45 female rats (Wistar strain) of 200 gram (g) body weight were used. The 45 rats were divided into one controlled group of 15 rats and 3 experimental groups of 10 rats in each group. In each experimental group, the rats were again divided into 2 subgroups of 5 rats each. Food, but not water, was deprived in the afternoon of the first experimental day. At 3.00 p.m. of the second experimental day, water was also withheld, and the animals were placed in individual cylindrical plastic

cages (PVC or Teflon tube). These cages were made with flat bottoms and with perforations to allow ventilation. The cages limited rat's movement and thus prevented ingestion of hair and feces. This procedure did not appear to be stressful (6), the animals were often observed to be asleep in such cages. On the third day at 9.00 a.m. the stomach ulcers were made by giving orally 1 millilitre of 0.6 N HCl necrotizing agent.

Thirty minutes before administration of the necrotizing agent, each rat of the controlled group was administered with 2 ml distilled water while the 3 experimental groups were administered with turmeric, straw fibers and lemon grass-fibers respectively.

The prevention action of turmeric against the ulcer was compared with the other two plant-fibers: straw-fiber and lemon grass-fiber. The turmeric powder was administered at doses of 62.5 milligram (gr. A) and 125 mg (gr. B) per rat of 200 g body weight. The solutions of turmeric were made by dissolving the powder in one millilitre of distilled water, and this procedure was done in the same way to the other two plant-fibers. The straw-fiber and the grass-fiber were used as placebo control. The straw-fiber was administered at doses of 15.625 mg (gr. C) and 31.25 mg (gr. D) per rat. The lemon grass-fiber *(Imperata arundinacea* Cyr.) was administered at doses of 19.16 (gr. E) and 38.32 mg (gr. F) per rat.

The weights of each dose (low dose and high dose) in these three categories (turmeric, straw-fiber and lemon grass-fiber) were not the same. However, their bulks were equivalent in size for each corresponding dose. The 2 ml volume of distilled water in the controlled group was equivalent to the volume of the fiber plant solution in the experimental groups.

The animals were sacrificed 1 hour after administration of the necrotizing agent. Their stomachs were dissected out, opened along the greater curvature and the mucosae were examined with a 2×binocular magnifier for the occurrenct of necrotic lesions and the number of which was counted.

The results were expressed as the average number and area of lesions per stomach and the percentage reduction from control of the ulcer number. Student's test was used to determine statistical significance.

RESULTS

1. Amount of gastric lesions produced by 1 ml of 0.6 N HCl in female rats

Table 1. shows the average number of lesions per stomach in each group. The controlled group received 2 ml distilled water. The six experimental groups received three categories of fiber-plants. Number of lesions was reported from the mean value of 5 rats. The number of lesions in the controlled group was 7.0 ± 1.58. The turmeric treated group (gr. A), which received 62.5 mg turmeric per rat, showed 3.2 ± 0.87 number of lesions. The turmeric treated group (gr. B), which received 125.0 mg per rat, showed 0.6 ± 0.55. The straw-fiber treated groups which received 15.625 mg and 31.25 mg (gr. C & D) showed 9.4 ± 1.52 and 8.8 ± 1.92 number of lesions respectively. The lemon grass-fiber treated groups which received 19.16 and 38.32 mg (gr E & F) showed 6.6 ± 1.14 and 6.4 ± 1.83 number of lesions respectively.

When comparing turmeric treated groups with distilled-water-treated controlled group, turmeric could significantly reduce the number of gastric lesions in rats. However, the straw and the grass fiber had no role in reducing the HCl-induced gastric lesions in our experiment. Moreover, a small dose of straw-fiber (gr. C) significantly produced more gastric ulcer lesions.

2. Area of ulcers produced by 1 ml of 0.6 N HCl in female rats

When taking the number of lesions and their sizes into consideration, i.e. computing into area, a more precise of value of real lesions was able to be expressed.

Table 2. shows the average area in square millimetre (mm^2) of gastric lesions per stomach in each group. Area of lesion was reported as the mean value of 5 rats. The mean area of ulcers in the controlled group was 57.2 ± 21.38 mm^2. The high (gr. A) and low (gr. B) dose turmeric treated group showed the mean values of 7.8 ± 5.85 mm^2 and 0.24 ± 0.18 mm^2 respectively. The straw-fiber treated groups, gr. C & D, showed the mean values of 86.0 ± 14.56 and 78.2 ± 14.22 mm^2 respectively. The grass-fiber treated groups, gr. E & F, showed the mean ulcer areas of 58.6 ± 5.73 and 56.6 ± 9.78 mm^2 respectively.

Both low and high doses of turmeric treated groups could significantly reduce the ulcer areas (table 2) as well as the number of gastric lesions (table 1) in this experiment. However, the two straw-fiber groups and the two lemon grass-fiber groups produced non-significant changes in the ulcer areas from the controlled group.

Table 1 Protection Effects of Turmeric, Straw-fiber Rice, Lemon Grass-fiber on Number of Gastric Lesions Produced by 0.6 N HCl in Female Rats

Item	Control	turmeric		straw		lemon grass	
Group		A	B	C	D	E	F
Number of Rats	15	5	5	5	5	5	5
Doses of plant fiber (mg.)	–	62.5	125.0	15.625	31.25	19.16	38.32
pH of solution	7.0	5.60	5.55	6.24	5.73	5.79	5.55
Average number of gastric lesions (ulcers) per stomach (X ± SD)	7.0 ±1.58	3.2 ±0.87	0.60 ±0.55	9..40 ±1.52	8.80 ±1.92	6.60 ±1.14	6.40 ±1.83
Statistical significance	–	S*	S*	S**	NS	NS	NS
t value	–	5.171	10.679	3.539	2.449	0.492	0.647
p value	–	< 0.01	< 0.001	< 0.05	> 0.05	> 0.5	> 0.5

S* = reduction of ulcer
S** = increase of ulcer
NS = not significantly different

Table 2 Protection Effects of Turmeric, Straw-fiber Rice, Lemon Grass-fiber on Area of Gastric Lesions Produced by 0.6 N HCl in Female Rats

Item	Control	turmeric		straw		lemon grass	
Group		A	B	C	D	E	F
Number of Rats	15	5	5	5	5	5	5
Doses of plant fiber (mg.)	–	62.5	125.0	15.625	31.25	19.16	38.32
Average area of gastric lesions (ulcers) per stomach (X ± SD) (mm^2)	57.2 ±21.38	7.8 ±5.85	0.24 ±0.18	86.0 ±14.56	78.2 ±14.22	58.6 ±5.73	56.6 ±9.78
Statistical significance	–	S	S	NS	NS	NS	NS
t value	–	4.611	5.932	2.763	2.039	0.134	0.054
p value	–	< 0.01	< 0.005	> 0.05	> 0.05	> 0.5	> 0.5

Table 3 Cytoprotection by Various Doses of Turmeric in Female Rats

Pretreatment agent	Average area of gastric ulcer per stomach (mm^2)	area reduction of ulcer (mm^2)	Percent reduction of ulcer area
H_2O	57.2 ± 21.38	–	–
Turmeric 31.25 mg (1/8 cap.)	32.0 ± 9.82	25.20	44.05
Turmeric 62.5 mg (1/4 cap.)	7.8 ± 5.85	49.40	86.36
Turmeric 125 mg (1/2 cap.)	0.24 ± 0.18	56.96	99.58
Turmeric 250 mg (1 cap.)	0.32 ± 0.16	56.88	99.44

3. Gastric cytoprotection by various doses of turmeric in female rats.

Since those two doses of turmeric showed dose-dependent manner, the experiment was thus extended to four doses in order to confirm the results of turmeric on prevention of gastric lesion. Comparing to the former controlled group, the outcome showed that the doses of 31.25, 62.5, 125 and 250 mg per rat yielded 44.05, 86.36, 99.58, 99.44 percent reductions of ulcer area respectively (table 3).

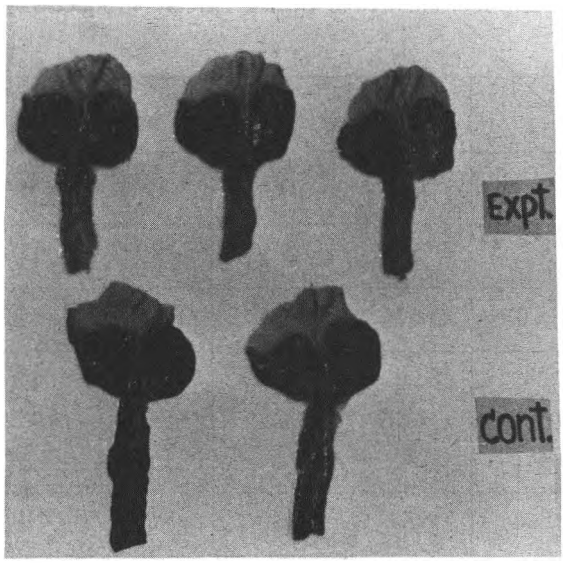

Figure 1 Illustration of HCl-induced gastric necrosis in turmeric pretreatment (Expt.) rats and controlled (Cont.) rats.

DISCUSSION

All experimental groups except two turmeric treatment groups showed marked gastric damage. After opening the stomach, lesions were found on the mucosa consisting of elongated bands, 1-10 mm long and 1-3 mm wide, usually parallel to the long axis of the stomach (figure 1). They were located mostly in the corpus (the portion of the stomach secreting acid and pepsin) while the antrum was less affected. No gross lesion developed in the forestomach (the nonsecretory part covered with a squamous epithelium).

We found that turmeric protect the stomach against necrotizing effect of HCl, while the other two plant fibers did not. The protection was as following, turmeric of dosages of 31.25 mg and 62.5 mg per 200 g body weight rat could reduce the ulcer formation by 44.05 percent and 86.36 percent respectively, while dosages of 125 mg and 250 mg showed approximately equal reduction by about 99.50 percent. This phenomenon represented gastric cytoprotection, since the gastric mucosal cells are protected against the effect of necrotizing agent.

The above findings are supported by the theory that cytoprotection is different from and unrelated to inhibition action of gastric secretion because cytoprotection, gastric and intestinal, is defined as property of substance to protect the mucosa of the stomach and the intestine from becoming inflamed and necrotic when this mucosa is exposed to noxious agents. (6, 7)

The pH of solutions of all fiber plants were between 5.5 to 6.2 which were not significantly different from each other. Therefore, the pH was unlikely to be related to either ulcerogenic effect or anti-ulcer effect. The result of inhibition of the HCl-induced ulcer did not depend on the pH of the solution. (table 1.)

Although the number of rats used in this experiment was only 5 per each experiment group, this number was acceptable. Goodwin (8) mentioned that careful planning and refinement of techniques could lead to reduction in the number of animals needed to obtain statistically significant answers. The "Three R's" rule, i.e. Refinement, Reduction and Replacement of experiments on animals, was laid down by WHO.

This is the first report of study about the cytoprotection of turmeric on the mucosa of stomach. The mechanism of gastic cytoprotection by turmeric is unknown. Several possible mechanisms must be further studied and collected. Another concurrent study was carried at the Department of Medical Sciences, Ministry of Public Health with various methods of producing ulcer in rats.

Turmeric could prevent gastric mucosa from noxious agents which caused acute and local aggressive ulcer. There was a clinical evidence which support this possibility. A 78 year old Thai man had gastroduodenal ulcer diagnosed by endoscopy. He had received anti-inflammatory drug for a week before the diagnosis. Two 600 mg turmeric capsules were given to him four times daily. Within 19 days, the ulcer was completely healed. This clinical report supported the animal experiment. Turmeric should be usefully prescribed in prevention and treatment of acute peptic ulcer due to localized noxious agents. However, there should be 15 more patients in an experiment to confirm the usefulness of the medication.

Turmeric is effective in treating peptic ulcer when given by mouth. The antiulcer property is probably due to a local action of turmeric since its effect is very rapid when given orally. Patients with duodenal ulcers usually have 40 percent spontaneous healing of ulcers within 4 weeks (5, 9, 10, 11). However, in the turmeric-treated patient, we found that the healing took place within 19 days of treatement. The rapidity of the action of turmeric when given by mouth, i.e. within only 19 days, not 4 weeks, suggested that turmeric probably has a local action in the stomach. A similar study of Robert (6) demonstrated that giving prostaglandins to rats orally caused more rapid effect than systemic administration of the drug. He explained that prostaglandins had a local effect as a cytoprotective which prevented the rat gastric mucosa from developing ulcers when given various local necrotizing agents. In his study, subcutaneous administration of prostaglandins produced a slower cytoprotective action than oral administration despite using a higher dose (4 times the oral dose) (6).

Several possible mechanisms of gastric cytoprotection have been discussed in an excellent review by Miller and Jacobson (12). From our experiment, few hypotheses could be cited. The first hypothesis explaining turmeric protecting effect on gastric mucosa against ulcerogen, might be the enhancement of the gastric mucus content. The release of mucus in animal after giving *Curcuma longa* (13) would explain the role in gastric epithelium protection. The surface mucous cells are capable of producing both mucus and bicarbonate; the two factors which seem to be of considerable importance in the phenomenon of cytoprotection (14, 15). It is also of interest to examine in man whether turmeric can increase the surface mucous cells and then increase mucus content which will render the gastric mucosa more resistant to noxious agents.

The second hypothesis is that turmeric might has a trophic effect on gastric mucosal barrier when used as local application. This hypothesis might be parallel to that Rienhart has done (16). He gave prostaglandin E_2 intragastrically to rat. The result was that there was an increase in connective tissue and mucous epithelial cell layer in all parts of the gastro-intestinal tract. This trophic effect was occurred by topical application. Also, this trophic effect increased both surface mucous cell and mucus content. Secretion of mucus provided a viscous physical barrier between the damaging agents and the surface epithelium (15).

The other mechanisms that might be worth studied are, the inhibition of gastric acid secretion, the effect on gastric mucosal circulation which can promote the cytoprotective property, and the direct effect on cell in increasing the intrinsic resistance of gastric mucosal cell to injury. Turmeric may raise tissue levels of prostaglandins in gastric mucosa and enhance cellular resistance of the gastric cells and thus the cells remain normal in the presence of strong irritants in the lumen (7, 17).

Cytoprotective turmeric may have therapeutic appli-

cations in many area. For examples, the turmeric may be beneficial in the treatment of acute gastritis caused by irritants, in gastric infection as there is a report of using this traditional medicinal plant as a remedy for gasto-intestinal diarrhoea (2), and may also be beneficial in reflux esophagitis. Turmeric may, not only accelerate the healing of peptic ulcers, but also prevent their recurrence.

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