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PHCOG MAG.: Research Article

Prokinetic Effect of Polyherbal Formulation on Gastrointestinal Tract

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ABSTRACT
PHF, a polyherbal formulation, consist of seven known herbs namely, *Aegle marmelos*, *Elettaria cardamomum*, *Glycyrrhiza glabra*, *Citrus aurantifolia*, *Rosa damascena*, *Cissus quadrangularis* and *Saccharum officinarum*. The PHF was evaluated for acute toxicity, gastrointestinal motility and gastric emptying rate in mice and rats. Based on acute toxicity study, the PHF was considered as safe and 3 dose (100, 200 and 400 mg/kg) levels were employed for further pharmacological studies. The gastrointestinal prokinetics effect of PHF in various dose levels (100, 200 and 400 mg/kg., *p.o*) was studied by charcoal meal gastrointestinal transit and laxative effect in mice. The gastric emptying rate of PHF in rat was studied by disappearance of phenol red from stomach. The results illustrate that PHF at 200 and 400 mg/kg significantly ($p<0.001$) enhanced the gastrointestinal transit. PHF at 400 mg/kg ($p<0.01$) significantly enhanced the purging index, which is the measure of laxative activity. PHF at 200 mg/kg ($p<0.05$) less significantly enhanced the purging index. In gastric emptying rate, PHF dose dependently increased the gastric emptying. From the above findings, PHF may be used as gastrointestinal prokinetics and to improve the intestinal motility.
KEY WORDS: Polyherbal formulation, prokinetics, gastrointestinal motility and gastric emptying.

INTRODUCTION

Gastrointestinal prokinetics promote the coordination of the gut wall contractions leading to enhancement of propulsive motility and consequently caudal displacement of luminal contents. Currently, they are considered drugs of choice for the treatment of upper gastrointestinal tract functional, motor disorders such as those associated with gastro oesophageal reflux disease, chronic dyspepsia and acute or chronic idiopathic intestinal pseudo obstruction. (1)

Although several chemicals and drugs are generally used against intestinal dysmotility and related intestinal diseases, the indigenous drugs with a long descended heritage of traditional use are of supreme importance, to re-establish traditional claims with scientific interest. Herbal medicines are obtained from various plants and contain complex extracts with a large number of different active substances. The combination of herbs with various gastrointestinal active ingredients appears to be advantageous for a heterogeneous condition such as functional dyspepsia, gastro oesophageal reflux disease and intestinal related disorders. In the present study, the prokinetic effect of polyherbal formulation (PHF) on gastrointestinal motor function was investigated. The herbal formulation used in this study consists of seven medicinal plants namely *Aegle marmelos*, *Elettaria cardamomum*, *Glycyrrhiza glabra*, *Citrus aurantifolia*, *Rosa damascena*, *Cissus quadrangularis* and *Saccharum officinarum*. The ethonomedical uses and biological activity of the medicinal plants present in the PHF is given in Table 1. The prokinetics activity of PHF was evaluated *in vivo* by intestinal transit rate of charcoal meal and laxative activity in mice and gastric emptying rate of phenol red in rats.

Table: 1. Ethnobotanical / Ethnopharmacological uses/ Biological activity of plants present in polyherbal formulation.

Plant name and Family	Parts used	Ethnobotanical / Ethnopharmacological uses/ Biological activity	References
Aegle marmelos Corr. Rutaceae	Roots, Leaves and Fruits	Dyspepsia, Stomachalgia, Gastric irritability, Digestive, Laxative	(2)
		Hypoglycaemic	(3)
		Anti ulcer	(4)
Elettaria cardamomum Maton. Zingeberaceae	Seeds	Carminative, Digestive, Stomachic, Dyspepsia, Gastropathy, Hyperdipsia	(2)
		Gastro protective activity	(5)
Glycyrrhiza glabra L. Papilionaceae	Roots	Emetic, Diuretic, Laxative, Aphrodisiac, Hyperdipsia, Cough, Bronchitis, Gastralgia, Gastric ulcers	(2)
Citrus aurantifolia Swingle. Rutaceae	Fruits	Laxative, Appetizer, Stomachic, Digestive, Anthelmintic, cough, Bronchitis, Dyspepsia, Flatulence, Helmenthiasis,	(2)
Rosa damascena Mill. Rosaceae	Flowers	Abdominal and Chest pain, Strengthening the heart	(6)
		Menstrual bleeding and Digestive problem	(7)
		Anti inflammatory activity	(8)
		Cough remedy	(9)
		Laxative	(10)
		Anti HIV	(11)
		Anti tussive	(12)
Saccharum officinarum Linn. Poaceae	Roots & Stems	Diuretic, Laxative, Cardiotonic, Aphrodisiac, Expectorant, Haemostatic, Dipsia, Gastropathy, Erysipelas	(2)
		Cholesteral Lowering effect, Inhibition of Platelet aggregation, Antioxidant activity	(13, 14, 15)
Cissus Quadrangularis Linn. Vitaceae	Whole Plant	Laxative, Anthelmintic, Carminative, Digestive, Stomachic, Aphrodisiac, Helminthiasis, Anorexia, Dyspepsia, Flatulance, Chronic ulcers, Tumours, Haemorrhoids, fractures etc.	(2)
		Gastro protective activity	(16)

MATERIALS AND METHODS

Plant material

Each gram of polyherbal formulation (PHF) contains powders of *Aegle marmelos* Corr. (Rutaceae; fruit, 150 mg), *Elettaria cardamomum* Maton. (Zingeberaceae; seed, 125 mg), *Glycyrrhiza glabra* L. (Papilionaceae; root, 150 mg), *Citrus aurantifolia* Swingle. (Rutaceae; fruits, 150 mg), *Rosa damascena* Mill. (Rosaceae; flower petals, 150 mg), *Cissus quadrangularis* Linn. (Vitaceae; whole plant, 150 mg) and *Saccharum officinarum* Linn (Poaceae; root, 125 mg). The plant materials were procured from a local supplier. Prof. R. Duraisamy, Botanist, authenticated the botanical identity of the plants and voucher specimen (NCP/Phcog/2008/0201) has been retained, for future reference, in the herbarium of the Pharmacognosy Department, Nandha College of Pharmacy, Erode, India.

Animals

Male Swiss albino mice weighing between 20 – 25 g and Wistar rats either sex weighing between 150 – 220 g were used for this study. The animals were obtained from the animal house, IRT Perundurai Medical College, Erode, Tamil nadu, India. On arrival, the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy

husk as bedding. Animals were housed at a temperature of 24±2 °C and relative humidity of 30 – 70 %. A 12:12 light: day cycle was followed. All animals were allowed free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (688/2/C-CPCSEA) and were in accordance with the Institutional ethical guidelines.

Acute toxicity study

Acute toxicity studies were performed according to OECD-423 guidelines (17). Male Swiss mice selected by random sampling technique were employed in this study. The animals were fasted for 4 h with free access to water. PHF was administered orally at a dose of 5 mg/kg initially and mortality if any was observed for 3 days. If mortality was observed in two out of three animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one animal out of three animals then the same dose was repeated again to confirm the toxic effect. If no mortality was observed, then higher (50, 300, 2000 mg/kg) doses of PHF were employed for further toxicity studies.

Charcoal meal gastrointestinal transit test

The method of Croci et al. was used with slight modifications (18). Mice (20–30 g) were divided into five groups of six mice each and fasted for 24 h before the experiment. Group I served as control with suspension of 1% CMC in distilled water (10 ml/kg, *p.o.*), group II was administered carbachol (1 mg/kg, *p.o.*), a standard cholinergic agent, as the positive control. Groups III - V were then treated orally with three increasing doses of the PHF, 100, 200 and 400 mg/kg. The drugs were administered by suspending in 1% carboxymethylcellulose solution. After 15 min, the animals were given 0.3 ml of freshly prepared charcoal meal (distilled water suspension containing 10% gum acacia, 10% vegetable charcoal and 20% starch). Following 30 min of charcoal administration, the mice were sacrificed by cervical dislocation and the abdomens immediately opened to excise the whole small intestine (pylorus region to cecum). The length of the small intestine and the distance between the pylorus region and the front of the charcoal meal was measured for obtaining the charcoal transport ratio or percentage.

Screening for laxative activity

Screening of laxative was based on an earlier reported method (19). Mice were fasted for 18 h and placed in individual observation cages, which were lined at the bottom with pre-weighed sheets of white absorbent paper. Six animals in individual cages were considered as a group and a total of 36 animals were thus divided into 5 groups. The first group served as the control. The second group received sennoside B (50 mg/kg, *p.o.*), the standard drug for comparison of potencies. Groups 3–5, received PHF, in doses of 100, 200 and 400 mg/kg. The drugs were administered orally by suspending in 1% carboxymethylcellulose solution. The animals were examined for hourly laxation for 5 h with the withdrawal of food and water. The percentage of respondents [*i.e.* (number of mice exhibiting laxation /total number of mice tested) X 100], latent periods (min) and number of wet feces passed during the test period were noted for each group and from the data, purging index was calculated. Laxation was evidenced by any increment of purging indices as compared to the control.

Gastric emptying rate (GER) of phenol red meals in rats

The GER was determined in rats by measuring the disappearance of phenol red from the stomach according to a previous method (20), with minor modifications. Various doses of PHF (100, 200 and 400 mg/kg body weight) were administered intra-gastrically to conscious rats. The drugs were administered orally by suspending in 1% carboxymethylcellulose solution. Mosapride (30 mg/kg) was used as reference standard. After 30 min, 1.5 ml of a phenol red meal, consisting of phenol red (0.05%, w/w) in 1.5% methylcellulose, was given to the rats intra-gastrically. Six rats were tested for each test drug. At 20 min thereafter, the rats were sacrificed by cervical dislocation, the abdominal cavity was opened, the gastro esophageal junction and the pylorus were clamped, and the stomach was then extirpated and rinsed in 0.9% saline. The stomach was placed in 100 ml 0.1 N NaOH, and homogenized. The suspension was allowed to settle for 1 h at room temperature, and 5ml of the supernatant was then added to 0.5 ml 20% trichloroacetic acid (w/v) and the suspension centrifuged at 3000 rpm at 4 ◦C for 20 min. The supernatant was mixed with 4 ml of 0.5 N NaOH, and the absorbance of the sample was read at 560 nm (A_{560}) using a U-3000 spectrophotometer (Hitachi, Ltd., Tokyo, Japan). The phenol red recovered from the animals that had been killed immediately after the administration of the methyl cellulose solution was used as the control (i.e., 0% emptying). The GER in the

20-min period was calculated according to the following equation.

GER (%) = {1 − (A_{560} of test/A_{560} of control)} × 100.

Statistical analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's *t* - test. P values <0.05 were considered significant.

RESULTS

Acute toxicity study

All the doses (5, 50, 300, 2000 mg/kg) of PHF employed for acute oral toxicity studies were found to be non-toxic. PHF did not produce any mortality even at the highest dose (2000 mg/kg) employed. Three sub maximal doses (100, 200 and 400 mg/kg), which were found to be safe, were employed for further pharmacological investigations.

Charcoal meal gastrointestinal transit test

Figure 1 shows the activity of the PHF to influence the charcoal meal gastrointestinal transit in mice. It was determined by its effect on the traverse of charcoal meal through the length of the small gut of mice. PHF dose-dependently propelled the charcoal meal travel through the small intestines. The distance traveled by the solvent control was 14.93±1.89%. The PHF at the dose of 100 mg/kg moved the charcoal meal to 20.97±2.99%, while 50.76±4.56% and 78.95±1.66% (P< 0.001) was caused with the next higher doses (400 mg/kg and 200 mg/kg, respectively). Carbachol (1 mg/kg) was used as positive control which moved the meal to 83.85±2.66% (P< 0.001).

Gastric emptying rate of phenol red meals in rats

The effect of PHF on gastric emptying rate of phenol red meal in rats is given in Figure 1. The % gastric emptying rate of phenol red meals during the 20 min period were 56.15±0.70% for control and 57.12±0.90, 61.87±2.12 and 70.82±1.32% respectively, for 100, 200 and 400 mg/kg doses of PHF in rats. The reference drug mosapride enhances the % gastric emptying rate up to 80.77±0.74 %. The % gastric emptying rate of phenol red meal was dose dependently enhanced by pretreatment with PHF suggesting that it has significant gastric emptying effect in rats.

Screening for laxative activity

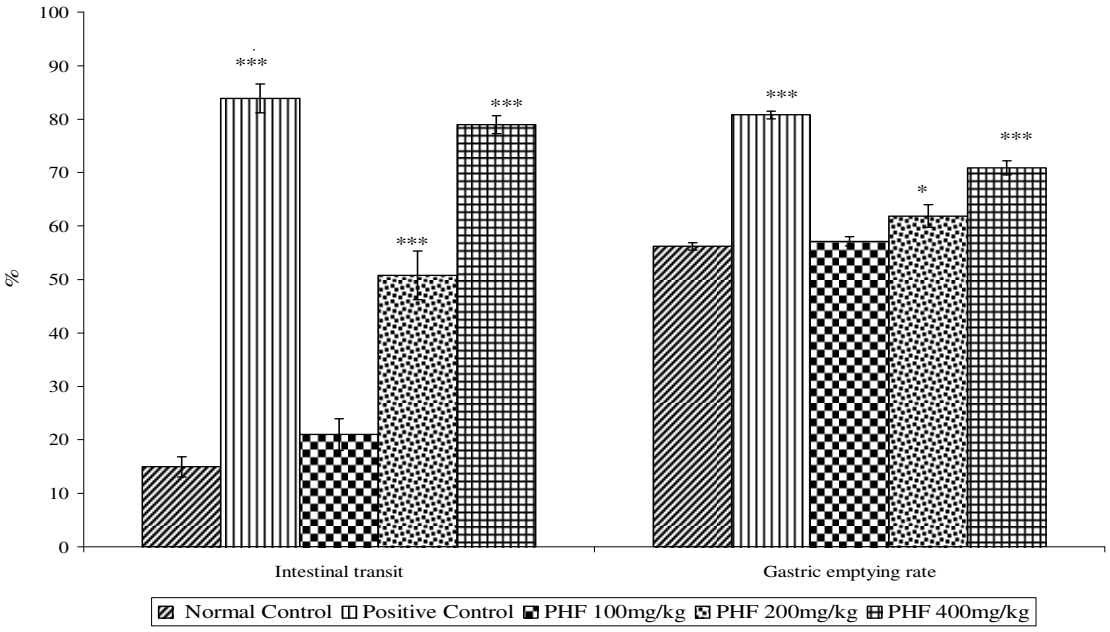
The animals were examined for hourly laxation for 5 h after the administration of test drugs and expressed as purging index (Figure 2). From the result of laxative screening, the PHF was found to exhibit laxative activity when administered at 400 mg/kg *b.w.* from 120 minutes onwards. Initially, there was no laxation and data obtained from 120 to 300 min were statistically significant (p< 0.01). The PHF at lower dose levels of 200 mg/kg *b.w.* also exhibited laxation but the results are less significant (p< 0.05) when compared to the control. The PHF at still lower dose levels of 100 mg/kg, b.w did not exhibited significant laxation. The PHF increased the wetness of faecal droppings at 400 mg/kg, *b.w.* The reference standard sennoside B exhibited significant (p<0.001) laxative property.

DISCUSSION

Propulsive motility is termed peristalsis and is subserved by a complex pattern of neural reflexes that aim to relax intestinal muscle downstream (descending inhibitory reflex) and contract the muscle upstream (ascending excitatory reflex) of the intestinal bolus. Intestinal transit is controlled by both neural and myogenic mechanisms (21). An increase of the contractile activity of the smooth muscle layers is in general responsible for acceleration of intestinal propulsion. Several mediators and neurotransmitters govern these motor patterns. Acetylcholine is the main excitatory neurotransmitter in the enteric nervous system, whereas NO is the major transmitter of the inhibitory motor neurons (22). Intestinal transit in the present experiment was enhanced by PHF treatment and may be mediated through cholinergic pathway. An increase of the contractile activity of the smooth muscle layers is in general responsible for acceleration of intestinal propulsion.

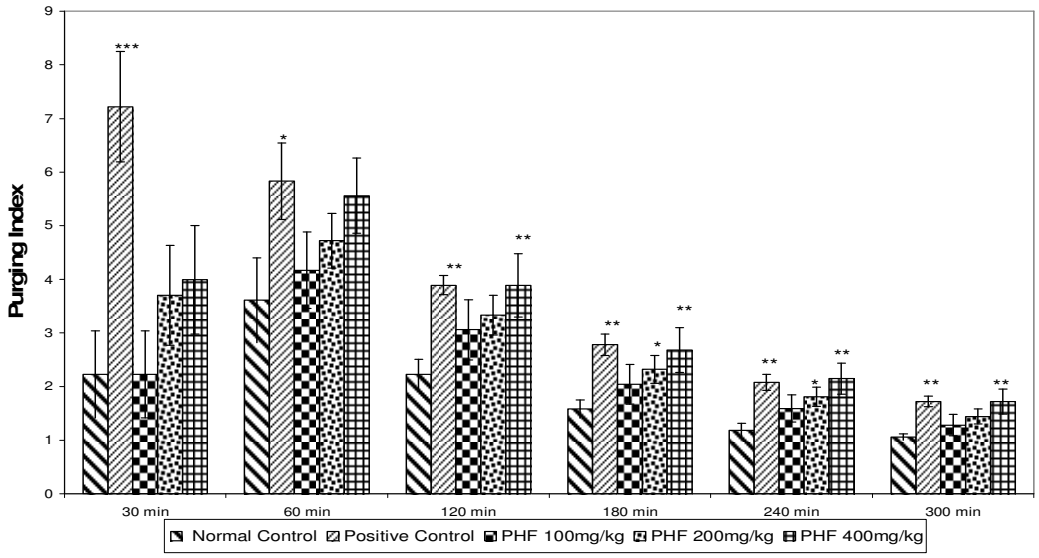
Gastric emptying is the process of the transfer of the gastric content to the small intestine as the result of the motor activity of the stomach, pylorus and duodenum under control of inhibitory and stimulatory mechanisms (23). The present findings demonstrate that % gastric emptying rate is dose dependently enhanced by PHF. Treatment of the animals with Mosapride increased rate of gastric emptying. The organization of gastric emptying is complex and involves the coordination of motor activity in the proximal stomach, the antrum, the pylorus and duodenum, as well as passive forces generated by intragastric volume and gravity (24, 25). The tonus of the sphincter is a determinant factor of the rate of gastric emptying (26). The enhanced effect of PHF may result from a prolonged relaxation of the pyloric sphincter.

Purging index is the measure of the extent of laxation. Increase in purging index is observed with increase in the transit of gastrointestinal tract. Carbachol, a cholinergic agent, enhance the purging index and wetness of faces which is a reliable marker for



***P<0.001 Vs control; **P<0.01 Vs control; *P<0.05 Vs control

Figure 1. Effect of PHF on the charcoal meal transit in mice and gastric emptying of phenol red in rats.



***P<0.001 Vs control; **P<0.01 Vs control; *P<0.05 Vs control

Figure 2. Effect of PHF on the purging index in rats.

increased gastrointestinal transit and secretion. Higher doses of PHF augment the frequency of defecation and propulsion of gastrointestinal tract.

CONCLUSION

In conclusion, PHF showed no acute oral toxicity in mice at a dose of 5 g/kg. The above results imply that PHF accelerates both the intestinal transit and gastric emptying; moreover the references support the anti-ulcer property of the herbs present in the polyherbal formulation. These observations suggest that PHF can be used as prokinetics and in the treatment of gastrointestinal motility disorders like gastro oesophageal reflux disease and chronic dyspepsia.

REFERENCES

1. M. Tonini. Recent advances in the pharmacology of gastrointestinal prokinetics. *Pharmacol. Res*. **33**: 217-26 (1996)
2. P.K. Warrier, V.P.K. Nambiar, C. Ramankutty. Indian Medicinal Plants. Vol. 1, (Oriant Longman Ltd., Arya Vaidya Sala, Kottakkal, 1994) pp. 31, 62, 84, 94, 112, 360.
3. A.Kar, B.K.Choudhary, N.G. Bhandyopathyay. Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. *J. Ethnopharmacol* **84**:105-108 (2003)
4. R.K.Goel, R.N. Maiti, M. Manickam, A.B.Ray. Anti-ulcer activity of naturally occuring pyranocoumarin and isocoumarin and their effect on prostanoids synthesis using human colonic mucosa. *Indian J Exp Biol*. **35**:1080-3. (1997)
5. M.A. Jafri, A. Jamal, K. Javed, M. Aslam. Gastroprotective effect of cardamom, *Elettaria cardamomum* Maton. fruits in rats. *J. Ethnopharmacol.* **103**: 149–53.(2006)
6. G. Wood, F. Bache. 1839. The Dispensatory of the United States of America, 4th ed. (Griggand Elliot, Philadelphia.1839) pp.232.
7. A. AveSina. Law in medicine. (Ministry of Guidance Publication, Tehran,1990) pp. 129–131.
8. J. Buckle. Clinical Aromatherapy in Nursing. (Arnold co-published by singular, London.1997) pp. 45
9. M. Libster. Delmars integrative herb guide for nurses. (Thomson Learing Alby, Delmar.2002) pp. 360–370.
10. A.Zargari. Medicinal Plants, vol. 2, 5th ed. (Tehran University Publications,Tehran. 1992) pp. 281–284
11. N. Mahmood, S. Piacenet, C. Pizza, A. Bruke, A. Khan, A. Hay. The anti-HIV activity and mechanisms of action of pure compounds isolated from *Rosa damascena*. *Biochem. Biophy. Res. Com*. **229**: 73–79.(1996)
12. M.N. Shafei, H. Rakhshandah, M.H. Boskabady. Antitussive effect of *Rosa damascena* in guinea pigs. *Iran. J. of Pharm. Res*. **2**: 231–234.(2003)
13. M.L.Arruzazabala, D. Carbajal, R. Mas, M. Garcia, V. Fraga. Effects of policosanol on platelet aggregation in rats. *Thromb Res.* **69**: 32 l-327. (1993)
14. M.L. Arruzazabala, D. Carbajal, R. Mas, V. Molina, S. Valdes, A. Laguna. Cholesterol-lowering effects of policosanol in rabbits. *Biol. Res*. **27**: 205–208. (1994)
15. M.L. Arruzazabala, S. Valdes, R. Mas, L. Fernandez, D. Carbajal. Comparative study of policosanol, aspirin and the combination of policosanol–aspirin on platelet aggregation in healthy volunteers. *Pharmacol. Res*. **36**: 293–297. (1997)
16. M. Jainu, K. Vijai Mohan, C.S.S. Devi. Protective effect of *Cissus quadrangularis* on neutrophil mediated tissue injury induced by aspirin in rats. *J. Ethnopharmacol* **104**: 302–305. (2006)
17. D. J. Ecobichon. The Basis of Toxicology Testing. (CRC Press, New York.1997) pp 43-86.
18. T. Croci, M. Landi, X. Elmonds-Alt, G. Le Fur, J.P. Maffrand, L. Manara. Role of tachykinins in castor oil induced diarrhoea in rats. *Bri. J. Pharmacol.* **121**: 375–380. (1997).
19. C.J.E. Niemeegers, F.M. Lenaerts, F. Awouters . Synthetic Antidiarrhoeal Drugs. (Marcel Decker. New York. 1976). pp. 65.
20. V. Martinez, S.V. Wu, Y. Tache. Intracisternal antisense oligodeoxynucleotides to the thyrotropin-releasing hormone receptor blocked vagal-dependent stimulation of gastric emptying induced by acute cold in rats. *Endocrinol.* **139**: 3730–735. (1998)
21. J. D. Huizinga, K. Ambrous , T. Der-Silaphet. Co-operation between neural and myogenic mechanisms in the control of distension-induced peristalsis in the mouse small intestine. *J Physiol*. **506**:843–56. (1998)
22. S.A. Waterman, M. Costa. The role of enteric inhibitory motoneurons in peristalsis in the isolated guinea-pig small intestine. *J Physiol***. 477**:459–68. (1994)
23. N.W. Weisbrodt. Gastric emptying. In: Johnson LR (Editor), Gastrointestinal Physiology. (St. Louis. Mosby. 1997). pp. 33-42.
24. M. Horowitz, J. Dent, R. Frazer. Role and integration of mechanisms controlling gastric emptying. *Dig Dis Sci*. **39**:7–13. (1994)
25. T. H. Moran, J.B. Wirth, W. G. Schwartz. Interactions between gastric volume and duodenal nutrients in the control of liquid gastric emptying. *Am J Physiol* **276**: 997–1002.(1999)
26. T. Ishiguchi, S. Nishioka, T. Takahashi. Inhibitory neural pathway regulating gastric emptying in rats. *J Auton Nerv Syst* **79**:45–51. (2000)

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