Effects of Juice from *Morinda citrifolia* (Noni) on Gastric Emptying in Male Rats

Hsiao-Fung Pu[1], Wei-Ju Huang[1], Wen-Min Tseng[1], Shyi-Wu Wang[2],Yu-Wen Liu[3], Ming-Long Doong[1], and Paulus S. Wang[1]*

[1]*Department of Physiology, School of Medicine*
National Yang-Ming University
Taipei 11221
[2]*Department of Physiology and Pharmacology*
Chang Gung University
Taoyuan 33333
and
[3]*Morinda International INC.*
Taiwan Branch, Taiwan, Republic of China.

Abstract

The effects of juice from *Morinda citrifolia* (noni) on gastric emptying, gastrointestinal transit, and plasma level of cholecystokinin (CCK) in rats were studied. Male rats were given noni by gavage at levels of 0.25, 1, or 4 ml/kg once per day for one or 7 days. The rats in the control group were given water, while the rats in the experimental group were fasted overnight before measurement of gastrointestinal motility. Gastrointestinal motility was assessed in rats 15 min after intragastric instillation of a test meal containing charcoal (10%) and $Na_2{}^{51}CrO_4$ (0.5 μCi/ml). Gastric emptying was determined by measuring the amount of radiolabeled chromium contained in the small intestine as a percentage of the initial amount received. Then, gastrointestinal transit was evaluated by calculating the geometric center of distribution of the radiolabeled marker. Finally, blood samples were collected for measurement of CCK by radioimmunoassay. The administration of noni at 0.25 ml/kg, but not at 1 ml/kg and 4 ml/kg, for 1 day significantly inhibited gastric emptying. In contrast, gastric emptying was significantly inhibited by oral noni (0.25, 1, or 4 ml/kg) for 7 days. Intraperitoneal injection of lorglumide (5 or 10 mg/kg), a selective CCK_1 receptor antagonist, effectively attenuated the noni-induced inhibition of gastric emptying. The intestinal transit and body weight, food intake, water intake, urine volume as well as feces weight were not altered by the administration of noni either acutely or chronically, but the administration of oral noni (1 ml/kg) for 7 days increased the level of plasma CCK in male rats. These results suggest that oral noni inhibits gastric emptying in male rats *via* a mechanism involving stimulation of CCK secretion and CCK_1 receptor activation.

Key Words: noni, cholecystokinin, gastric emptying, gastrointestinal transit, lorglumide

Introduction

The *Morinda citrifolia* L. plant and its fruit have long been an important medicinal product for the people in Pacific islands (1). The juice of Morinda is known as noni in Hawaiian and has been reported to have broad therapeutic effects, including antiviral, antibacterial, antifungal, antihumor, antihelmin, analgesic, hypotensive, anti-inflammatory and immune enhancing effects (33). However, the mechanisms of these effects remain unknown.

The function of CCK includes stimulation of pancreatic enzyme secretion and inhibition of gastric emptying (6, 9, 15), as well as suppression of food intake (24). Two subtypes of CCK receptors, CCK_1 and CCK_2, have been reported (25). CCK_1 receptors are found mainly in peripheral tissues and CCK_2 receptors express mainly in the central nervous system

Corresponding author: Dr. Paulus S. Wang, Department of Physiology, School of Medicine, National Yang-Ming University, Taipei 11221, Taiwan, Republic of China. Tel: 886-2-2826-7082, Fax:886-2-2826-4049, E-mail: pswang@ym.edu.tw
Received: May 7, 2004; Accepted: May 22, 2004.

(13, 16, 27, 29, 34). A number of studies have suggested that CCK_1 receptors play an important role in the inhibition of gastric emptying by amphetamine or endogenous and exogenous CCK (3, 7, 31); however, the mechanism by which oral noni inhibits gastric activity through CCK is unknown.

The purpose of this study was to investigate [1] the effects of oral noni on gastric emptying, intestinal transit, and plasma CCK levels, and [2] the involvement of CCK receptors in the action of oral noni on gastrointestinal (GI) motility in male rats by using antagonists of CCK_1, lorglumide.

Materials and Methods

Animals

Male Sprague-Dawley rats weighing 250-350 g were housed in a temperature (22±1°C)- and light (6 a.m. -8 p.m.)-controlled environment and fed with rat chow. Tap water was given *ad libitum*.

Animal protocols were approved by the Institutional Animal Care and Use Committee of the National Yang-Ming University. All animals received human care in compliance with the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals, published by the National Science Council, Taiwan, ROC.

Experiment 1. Chronic Effects of Noni Administration on GI Motility

Male rats were randomly divided into four groups and fed with noni (0.25 ml/kg, 1 ml/kg, or 4 ml/kg) *via* gavage administration once daily for 7 days. Noni was provided by Morinda International Inc., Taiwan Branch. The control rats were fed with tap water. All rats were housed in the Nalgene metabolic cages (Nalge Company, Rochester, NY, USA). The body weight, food intake, water intake, urine volume, and feces weight of each rat were recorded daily. Rats were fasted (with access to water) for 24 h before gastric intubation of a non-nutrient liquid meal. Fifteen min after the administration of the liquid meal, the rats were decapitated, and GI transit was measured. Blood samples were collected for CCK radioimmunoassay (RIA).

Experiment 2. Effects of Lorglumide on Noni-Mediated Inhibition of Gastric Emptying

Male rats were divided into four groups and fasted for 24 h before use. Fifteen min before gastric intubation of a non-nutrient liquid meal, the animals were injected i.p. with the following compounds in 1 ml/kg: group 1 received dimethyl sulfoxide (DMSO, Wako Pure Chemical Industries Ltd, Japan), while groups 3 and 4 received DMSO containing lorglumide (a CCK_1 receptor antagonist, Research Biochemicals International Company, Natick, MA, USA) at doses of 5 and 10 mg/kg, respectively. Groups 2-4 received 1 ml/kg of noni orally once daily for 7 days.

Experiment 3. Acute Effects of Noni Administration on GI Motility

The procedure was identical to that in experiment 1, except that the oral treatment of noni was performed for one day only.

Measurement of Gastric Emptying and GI Transit

Gastric emptying and intestinal transit were measured as described by Doong *et al*. (7). Rats were intubated *via* a catheter (PE-205, ID 1.67 mm, OD 2.42 mm, Clay-Adam, Parsippany, NJ, USA) with physiological saline (3 ml/kg) containing $Na_2{}^{51}CrO_4$ (0.5 μCi/ml) and 10% charcoal. The test meal was continuously stirred before intubation. Air (0.5 ml) was injected to flush the residual charcoal suspension in the catheter into rat stomach. Fifteen min later, the rats were decapitated and the stomach with the attached small intestine was immediately exposed by laparotomy. After ligation of the esophagogastric, gastroduodenal, and ileocaecal junctions, the whole stomach with the attached small intestine was carefully removed and placed on a wooden board to observe the leading edge of the charcoal in the intestine. The small intestine was then divided into 10 equal segments, and the radioactivity in the stomach and each segment of small intestine was measured in an automatic gamma counter (1470 Wizard, Pharmacia, Turku, Finland). Gastric emptying was measured by determining the amount of labeled chromium contained in the small intestine 15 min after intubation, expressed as a percentage of the amount given (4, 7, 10). Intestinal transit was assessed by calculating the geometic center of distribution of the radioactivity within the 10 segments (4, 21) by summation of the radioactivity in each segment multiplied by the segment number.

Processing of Plasma

After decapitation , rat blood samples were collected and mixed with EDTA (1 mg/ml of blood) and aprotinin (500 KIU/ml of blood). Plasma was immediately prepared by centrifugation at 1000 × g for 30 min at 4°C and used for measurement of plasma CCK concentrations. The plasma samples were acidified with an equal volume of 1% trifluoroacetic acid (TFA), and then centrifuged at 2600 × g for 20

min at 4°C. The SEP-PAK C18 cartridge (Waters Associates, Milford, MA, USA) was equilibrated with 60% acetonitrile in 1% TFA (1 ml), followed by 1% TFA (3 ml, three times). Then the supernatant from the treated plasma sample was applied. After slow washing with 1% TFA (3 ml, twice), the peptide (bound material) was slowly eluted with 3 ml of 60% acetonitrile in 1% TFA. The eluant was collected, lyophilized in a Speed Vac concentrator (Salvant Instruments, Farmingdale, NY, USA), and then stored at -80°C and reconstituted with the appropriate assay buffer before RIA (7, 12).

CCK Radioimmunoassay

The CCK concentration in extracted sample was measured by RIA using a rabbit anti-CCK antiserum supplied by Dr. K. Y. Francis Pau (Division of Reproductive Science, Oregon Regional Primate Research Center, Beaverton, OR, USA), and ^{3}H-CCK purchased from Amersham International Plc. (Bucks, UK). In this RIA system, a known amount of unlabeled CCK in a total volume of 0.3 ml of 0.1% gelatin-PBS was incubated at 4°C for 24 h with 100 µl of anti-CCK antiserum, diluted 1: 2,000 in normal rabbit serum, and 100 µl of [^{3}H]CCK (~8,000 cpm). Triplicate standard curves with 6 points ranging from 1 to 1,000 pg of unlabeled CCK were included in each assay. Two hundred µl of anti-rabbit gamma-globulin (ARGG) was then added and the incubation continued at 4°C for 24 h. The assay tubes were then centrifuged at 1,000 × g for 20 min. The pellet was dissolved in 400 µl of 1N NaOH. Then 80 µl of 5 N HCl was added, and the sample was mixed with 3 ml of liquid scintillation fluid. The radioactivity was counted in an automatic counter (Wallac 1409, Pharmacia, Turku, Finland). The sensitivity of the CCK RIA was 8 pg of CCK per assay tube. The intra-assay and inter-assay coefficient of variation were 3% and 5%, respectively.

Statistical Analysis

The data were expressed as the mean value ± S.E.M. The treatment means were tested for homogeneity using one-way analysis of variance, and the significance of any difference between means tested using Duncan's multiple range test (30). A difference between two means was considered to be statistically significant when *P* was less than 0.05.

Results

Effects of Noni Administration on Metabolism in Rats

The administrarion of noni (0.25, 1, and 4 ml/kg) *via* gavage for one day or 7 days did not alter the body weight, food intake, water intake, urine volume and feces weight (Figs 1. and 2). The water intake and feces weight were reduced following the one-day fast.

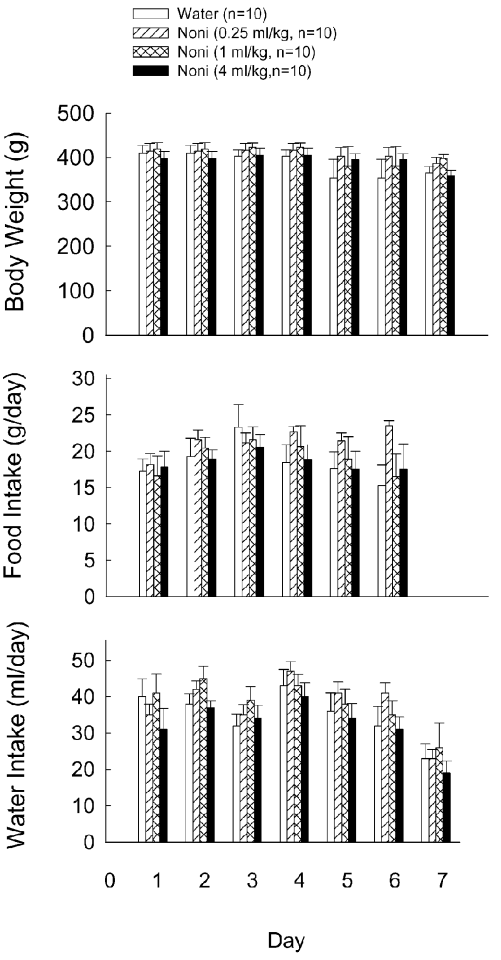

Fig. 1. Body weight, food intake and water intake by male rats fed with or without noni (0.25, 1, or 4 ml/kg) once daily for 7 days. The control rats were fed with water only.

Acute Effects of Oral Noni on Gastric Emptying and Intestinal Transit

Gastric emptying, but not intestinal transit, in male rats decreased (P<0.01) following oral ingestion of 0.25 ml/kg noni for one day (Fig. 3). Neither gastric emptying nor intestinal transit was altered by oral ingestion of 1 or 4 ml/kg noni for one day.

Chronic Effects of Oral Noni on Gastric Emptying and Intestinal Transit

Gastric emptying was reduced by 27% (P<0.05) in male rats following the oral administration of 0.25 ml/kg noni, and by 42-44% (P<0.01) following the

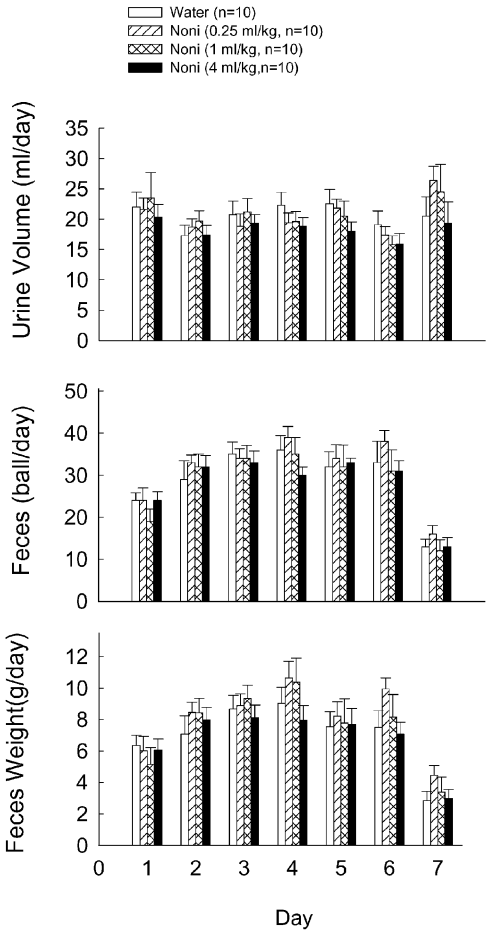

Fig. 2. Urine volume and feces weight excreted by male rat fed with or without noni (0.25, 1, or 4 ml/kg) once daily for 7 days.

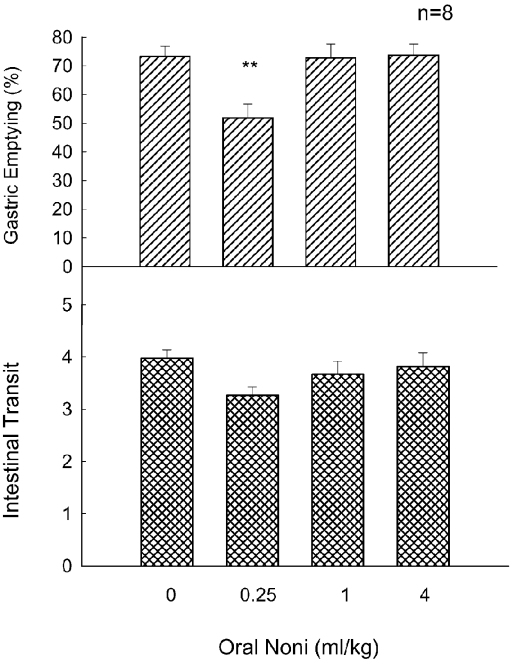

Fig. 3. Gastric emptying and intestinal transit in male rats following once ingestion of noni (0.25, 1, or 4 ml/kg) via gavage 24 h before measurement of gastrointestinal motility. Each column represents the mean ± S.E.M. $^{**}P < 0.01$ as compared with vehicle group (noni = 0 ml/kg).

administration of 1 or 4 ml/kg noni (Fig. 4, upper panel). Intestinal transit was reduced by oral administration of noni of 1 ml/kg, but not altered by that of 0.25 or 4 ml/kg noni (Fig. 4, lower panel).

Effects of Lorglumide on Noni-Induced Inhibition of Gastric Emptying

Treatment of lorglumide (5 or 10 mg/kg) significantly prevented ($P< 0.01$) the noni-induced inhibition of gastric emptying (Fig. 5, upper panel), and yet the inhibition of intestinal transit caused by noni-induced was not altered by the treatment of lorglumide.

Chronic Effects of Oral Noni on the Level of Plasma CCK

The oral administration of 1 ml/kg noni for 7 days significantly reduced the gastric emptying (Fig. 6, upper panel) but increased the level of plasma CCK (Fig 6, lower panel).

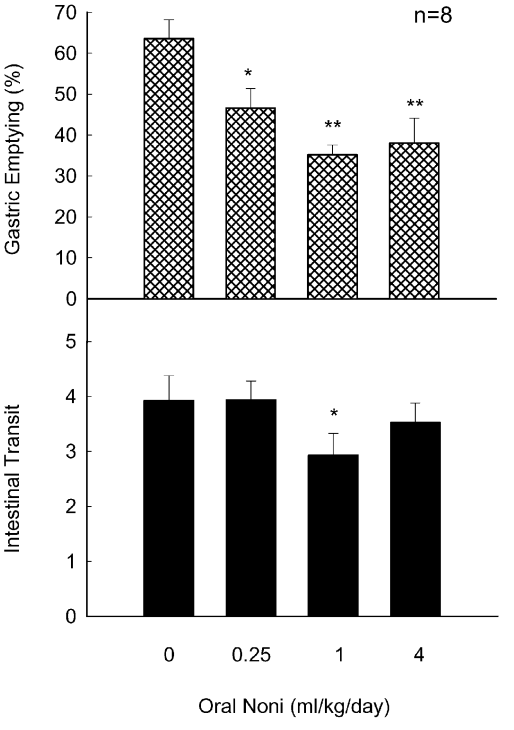

Fig. 4. Gastric emptying and intestinal transit in male rats following once ingestion of noni (0.25, 1, or 4 ml/kg) *via* gavage daily for 7 days before measurement of gastrointestinal motility. Each column represents the mean ± S.E.M. $^{*,**}P < 0.05$ and $P < 0.01$ as compared with vehicle group (noni = 0 ml/kg).

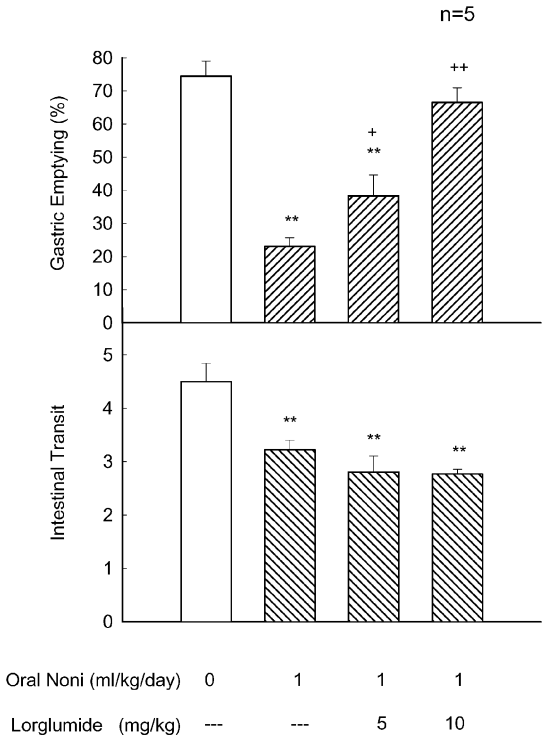

Fig. 5. Effects of lorglumide (a CCK_1 receptor antagonist) on the oral noni-inducd inhibition of gastric emptying (upper panel) and intestinal transit (lower panel) in male rats following administration of noni for 7 days. Each column represents the mean ± S.E. M. $^{**}P < 0.01$ as compared with vehicle group (noni = 0 ml/kg).

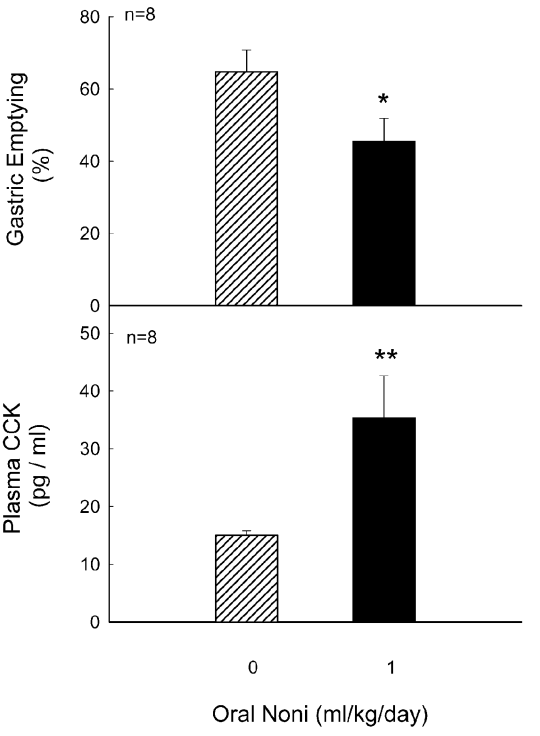

Fig. 6. Gastric emptying and plasma levels of CCK in male rats following administration of noni (1 ml/kg) once daily for 7 days *via* gavage before decapitation. Each column represents the mean ± S.E.M. $^{**}P < 0.01$ as compared to vehicle-injected rats.

Discussion

These results demonstrate that [1] the administration of noni inhibited gastric emptying but increased the plasma CCK concentration in male rats, and [2] the selective CCK_1 receptor antagonist, lorglumide, blocked the noni-induced inhibition of gastric emptying.

Noni has been reported to have a broad range of therapeutic effects including antiviral, antitumor, hypotensive, and anti-inflammatory effects (8, 23). The anticancer activity of noni has been attributed to the prevention of carcinogen-DNA adduct formation and the antioxidant activity of noni (32). Recently, it has been found that noni is highly effective to inhibit angiogenesis in human breast tumors (11). In the present study, we found that oral noni in a range of 0.25 - 4 ml/kg decreased gastric emptying but did not alter intestinal transit in male rats. Since the food intake, water intake, urine volume, and feces weight were not altered by the administration of noni, the change of gastric emptying caused by oral noni was independent of metabolic processes in rats.

It has been well known that CCK slows gastric emptying in both animals and humans (2, 5, 6, 14, 18). CCK suppresses food intake by inhibiting gastric emptying (24, 28). In the present study, oral noni of male rats resulted in an increase in the plasma CCK level and a marked decrease in gastric emptying induced by noni might be related to hypersecretion of CCK. Therefore we initiated the CCK antagonist trial. There is now a lot of evidence showing that selective CCK_1 receptor antagonists are able to counterbalance the effects of both exogenous and endogenous CCK (15, 17). CCK delays gastric emptying of liquids by stimulation of CCK receptors (3, 23, 31). It is also suggested that CCK inhibits gastric emptying in rats by causing contraction of the pyloric sphincter, which is prevented by CCK_1 receptor antagonists (19, 26). However, CCK_1 and CCK_2 receptor mRNAs have been detected in the rat stomach (22) and the role of CCK_2 mediating gastric motility has not been established. Our data show that lorglumide blocked the noni-induced inhibition of gastric emptying. Apparently, both the actions of CCK and CCK_1 receptor are involved in the regulation of noni on gastric emptying.

In summary, the present investigation suggest that oral administration of noni inhibits gastric emptying, which occurred concomittently with an increase of plasma CCK concentration. The results

also suggest that CCK_1 receptors are involved in the noni-induced inhibition of gastric emptying. These observations are consistent with the concept that noni, in association with CCK, plays important roles in the regulation of gastric motility.

Acknowledgment

The present study was supported by a research grant from Morinda International Inc., Taiwan Branch.

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