

Influence of hippophae rhamnoides on two appetite factors, gastric emptying and metabolic parameters, in children with functional dyspepsia

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Abstract

Our aim was to explore in children with functional dyspepsia the effect of hippophae rhamnoides on the levels of plasma appetite factors and on their gastrointestinal motility. *A hundred and twenty children* with functional dyspepsia were randomly divided into three groups: Group I (treated with hippophae rhamnoides), Group II (treated with domperidone), and Group III (treated with hippophae rhamnoides plus domperidone). The treatment lasted for eight weeks. The levels of plasma leptin (LP) and neuropeptide Y (NPY) were measured before and after treatment. All patients underwent a gastric emptying (GE) test by ultrasound (US) to measure the rate of postprandial gastric antrum residual, at 30min, 60min, 90min and 120min. The average value of subcutaneous fat, body fat percentage, upper arm girth and body mass index (BMI) were also measured. To compare the US with the radionuclide GE test 14 healthy adults volunteers were tested by both GE techniques. *We found* that the levels of LP and NPY in plasma were markedly higher after treatment in Groups I and III than in Group II. The postprandial gastric antrum remains at 60min, 90min and 120min in Groups I and III fell greatly and the thickness of skin fold (SF), body fat percentage and arm girth increased ($P < 0.05$). The GE half emptying time of a mixed liquid-solid food measured by B US and by the radionuclide technique in the same individuals was similar ($P > 0.05$). *In conclusion*, in children's functional dyspepsia, our study showed that hippophae rhamnoides increases the levels of appetite factors, leptin and neuropeptide Y, increases gastric emptying and gastrointestinal digestive function, children's growth and development.

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Introduction

Hippophae rhamnoides, commonly known as acid thorn or acid willow, is rich in bioactive components as vitamins, amino acids, organic acids, lipids, flavonoids and 5-hydroxytryptamine (5-HT) [1, 2] and among other useful affects in men can help increase the secretion of animal saliva, of the gastrointestinal glands and of protease in the stomach thus supporting food digestion [3].

Children's functional dyspepsia (CFD) refers to continuously repeated abdominal distention, feeling of fullness, anorexia and sickness [4]. The incidence of CFD was found to be up to 31% among children playing in the kindergartens of Liwan District in Guangzhou City, China. This disease greatly affects children's growth and development and raises serious attention in the medical circles at home and abroad. However, few reports have been found on the relationship between appetite regulators and gastrointestinal peristaltic ability in children with CFD, and there is no information in medical bibliography about the use of hippophae rhamnoides dry emulsion in treating anorexic children.

Children FD is clinically common but difficult to treat and has a complex pathogenesis. It is usually connected with bad eating habits, abnormal appetite factors, leptin (LP) and neuropeptide Y (NPY) deficiency of rare elements, rickets and gastrointestinal motility dysfunction parasitic diseases. Among the above factors, abnormality of appetite factors and disorder of gastrointestinal motility have been increasingly studied as etiologic factors of the disease. Emphasis is laid on the effect of the appetite, factors although this ignores the regulation of the brain-gut peptide-appetite center on eating behavior. Disorders of the brain-gut peptide-appetite center are closely related to the occurrence and development of CFD. The relationship between appetite factors and gastrointestinal motility, however, requires further research. Our present study is about a new herbal medicine extracted from a plant called hippophae rhamnoides, and whether this new herbal drug may influence LP, NPY and gastrointestinal motility.

To explore the function of hippophae rhamnoides dry emulsion in regulating the relationship between the levels of appetite factors and gastrointestinal motility in children with FD, we undertook the present study.

Subjects and methods

Subjects

A hundred and twenty children with FD diagnosed at the outpatients' department of our hospital from Jun 2009 to July 2011 have been studied. Among them 77 were boys and 43 girls, ranging from 1-10 years old. They were divided equally into three groups: Group I, treated with hippophae rhamnoides dry emulsion. There were 40 children in Group I, 27 boys and 13 girls, aged from 11 months to 9 years. There were also 40 children in Group II, 28 boys and 12 girls, aged from 15 months to 9 years. There were also 40 children in Group III, 22 boys and 18 girls, aged from 11 months to 10 years. There was no significant difference in age, sex and conditions among the three groups. Groups I and III were treated with hippophae rhamnoides and with hippophae rhamnoides plus domperidone, respectively, while Group II was treated only with domperidone.

Fourteen healthy adults volunteers free from any history or present symptoms related to digestive system or to any disease affecting gastrointestinal motility were also studied (Group IV). There were 8 men and 6 women in Group IV with mean age: 38.42 ± 14.23 years (range 20-65 years).

Diagnostic criteria for functional dyspepsia were [4]: a) continuously repeated pain or feeling of upset in the upper abdomen, b) no relief of symptoms after defecation or a change in the frequency of defecation or of its nature, c) no evidence of inflammation, organic diseases, metabolic diseases or tumors to explain for the symptoms and d) occurrence of symptoms at least once a week and lasting for more than 2 months.

Methods

Medication method: Hippophae rhamnoides dry emulsion (with its juice and oil), made by Shaanxi Haitian Pharmaceutical Company, Ltd., China, Batch No.: Guoyaozhunzi: B20021064, was given to patients aged 1~3 years twice a day in a dose of 5g, for those aged 4~6 years old, 10g, and for those aged 7 years old, 15g. Domperidone was produced by Xian Yangsen Pharmaceutical Company Ltd., China, Batch No.: Guoyaozhunzi: H10910003 and administered in a dose of 0.3mg/kg, three times per day. All treatment courses lasted for 8 weeks.

Blood plasma for leptine (LP) determination: Two mL of venous blood taken in the morning from patients with empty stomach was poured immediately into test tubes with EDTA and centrifuged at 3000r/min for 10min. Plasma was frozen at 20 degrees below zero for later determination. The LP level was determined by ELISA, with reagents made by Shenzhen Joyful Abundant Company, Shenzhen City, China, the steps of operation followed the Instructions suggested by the producer.

Blood plasma neuropeptide Y (NPY) determination. Two mL of venous blood was taken in the morning from the patients having empty stomach and immediately poured into test tubes with 30 μ L of 7.5% EDTA and 40 μ L of aprotinin. Tubes were evenly shaken before being centrifuged at 3000r/min for 10min and plasma was frozen at 70 degrees below zero for later use. The NPY of plasma was determined by radioimmunoassay (IRMA), with reagents made by the Eastern Asia Immune Institute of Technology of Beijing, China, PLA General Hospital (Beijing Poore Biotechnology Company Ltd.), the steps of the testing procedure followed the instructions of the producer.

The gastric emptying (GE) test was first performed by the B ultrasound (US) technique. This was carried out with an US scanner LOGIG3 made in the U.S.A. and by 3.5~5.0MHs probes. All subjects were asked not to take no medicine for the last three days before and not to take any food or drinks after ten a.m. the last day before the test. Real-time US imaging of the longitudinal section of gastric antrum was taken to check how the content of the stomach emptied. The subjects lied supine and real-time imaging was used to make sure that their stomachs were empty. The xiphoid, the left liver lobe and the aorta were marked on the skin of the tested subjects in order to estimate the area of gastric relaxation before the test meal was taken. This meal consisted of pure milk, 150mL for patients aged 1~3 years, 200mL for patients aged 3~5 years, and 250mL for those aged 5~7 years and was taken within 3min. Then the upper and lower diameters (A) of the longitudinal section of the gastric antrum and its anteroposterior diameters (B) were taken respectively at 3, 30, 60, 90 and 120min after the test meal. The area of the gastric antrum was considered an ellipse and calculated accordingly [5]: $S = \pi AB/4$. [$S^1 =$ the area of the gastric antrum at a certain time after taking the test meal/ $S =$ the area of the gastric antrum 3min after the meal] $\times 100\%$ (π is ratio of the circumference of a circle to its diameter, $\pi \approx 3.141592654$).

Method for measuring skin thickness: This was done by a thickness meter made by Jiangyin City Zhouzhuang Electric & Hardware Plant, China. Body mass index (BMI), calculated by weight (kg)/height(m²), upper arm girth and body fat percentage (male body fat percentage=6.931+0.428x; female=7.896+0.458x, "x" was triceps skinfold plus subscapular skinfold) were measured [6] and compared to the values of one or more normal subjects of the same age.

Comparison of GE half time by B US and by the radionuclide technique in the same subjects (Group IV)

We used a mixed solid-liquid meal; 60g cooked noodle, 60g fried egg without oil, 6g plant oil, 300mL boiled water and 64MBq ^{99m}Tc-phytate as a tracer for the solid and 18.5MBq of indium-113m diethylene triamino pentaacetic acid (^{113m}In-DTPA) to label the water of the meal. The total caloric value was 345kcal. The meal contained 18% proteins, 18% fat and 64% carbohydrates. The stability of the meal in vivo was tested.

The antral area measured by US at fast was defined as 0%. The postprandial maximal antral area was defined as 100%. The antral area at each measured area-point was converted to percent and called: the antral dilation rate and after correction for decay, the count at each measurement was converted to percent of the initial activity (IA) which was called: the gastric residual rate. A power exponential curve was used to analyze these data. The time that healthy subjects spent to empty all test meal was used to compare data.

Statistics

The SPSS13.0 statistical software was adopted to work out the results, and \pm SD was applied to indicate the measured data. Comparison of the physical indicators in each group before and after treatment was done with Student's *t* test, and the assessment of the effect, with the chi-square test. $P < 0.05$, was considered as statistically significant. We also used: Elashoff's non linear power index regression mode [7] and analysis of the intragastric food residue rate measured on each area-time by the B US and by the radionuclide GE technique.

Table 1. Measured value (ng/L) of LP and NPY levels before and after treatment in children with functional dyspepsia in the three groups

Groups	n	LP		NPY	
		Before treatment	After treatment	Before treatment	After treatment
Group I	40	107.52±24.12	137.38±29.86*	327.80± 67.56	501.04±155.49*
Group II	40	114.63±17.95	121.43±13.78	327.55± 121.25	375.92±101.35
Group III	40	114.56±25.10	149.27±31.99*	335.91± 69.84	525.29±152.70*
F value		13.25		15.12	
P		<0.05		<0.05	

F is the comparison of increased values among groups before and after treatment; *shows what is compared with that before treatment has P<0.01.

Table 4. The relative analysis of the GE time and gastric residual rate measured by US and the nuclear medicine technique in 14 healthy adults.

Comparative index	N	US	Nuclear Medicine techniques	r	P
R ₈₇	14	102±7.42	98.57±8.16	0.56	0.038
R ₈₇	14	63±16.00	61.00±19.00	0.52	0.047
R ₁₁₇	14	44±20.00	37.00±8.16	0.61	0.019

T_{1/2}: half emptying time; R87: postprandial gastric residual rate at 87min; R117: postprandial gastric residual rate at 117min.

Results

Comparison among Groups I, II and III patients showed that the levels of LP and NPY in plasma were remarkably higher after treatment in Groups I and III the difference being statistically significant (P<0.05), while in Group II these parameters did not show any statistical difference from their values before treatment (P>0.05) (Table 1).

Comparison between the 3 Groups of the % value of gastric residual rate after the test meal before and after treatment showed that remains in Groups I and III were much less than in Group II at 60min, 90min and 120min after taking the test meal, and that these differences were of statistical significance (P<0.05), while in Group II before and after treatment there was no such statistical significance (P>0.05), as indicated in Table 2.

The average thickness of skin fold (SF), in upper arm girth and body fat percentage in Groups I and III were much higher after treatment, the differences being remarkably significant (P<0.05), while no significant difference was measured before and after treatment for BMI (P>0.05, Table 3). The above three parameters as compared between Groups I and III before treatment showed no statistical difference. The same similarity was shown in the above values, after treatment.

Comparison among GE half time of mixed liquid-solid meal measured by real time US and by the nuclear medicine technique in the same individuals showed that both tech-

niques after postprandial 40min gave similar results as indicated in Figure 1.

There was no significant difference (P=0.16) between the GE half time of mixed liquid-solid food measured by B US (UT1/2) being 102.07±7.41min and the GE half time of mixed food measured by the nuclear medicine technique being 98.57±8.16min. Both results were in linear correlation (r=0.56, P=0.038). The mean postprandial gastric residual rate at 87min measured by B US (R₈₇) was 63%±16% and the same measured by the nuclear medicine technique was 61%±19%. There was no significant difference between these rates (P=0.31). Both tests were in linear correlation

in the 14 healthy adults volunteers (r=0.52, P=0.047). The postprandial gastric residual rate at 117min measured by B US was 44%±20%. The postprandial gastric residual rate measured by nuclear medicine technique was 37%±24%. There were no significant (P>0.15) difference of these rates between the two techniques, which were in linear correlation (r=0.61, P=0.019) as is indicated in Table 4.

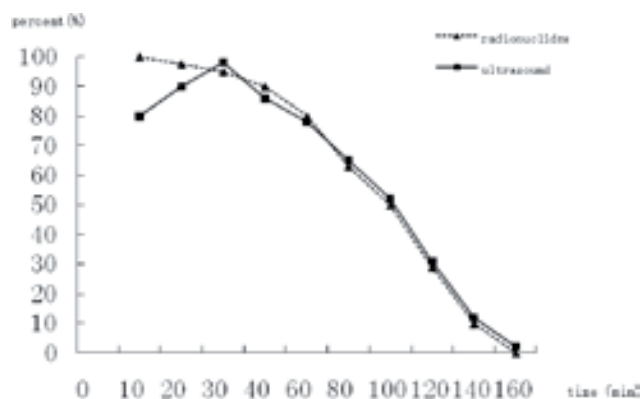


Figure 1. The curve of the gastric emptying of mixed food measured by ultrasonnd and radionuclides in 14 healthy adults.

Figure 1. The GE time-curve vs percentage of mixed food remaining in the stomach measured by US and nuclear medicine techniques in 14 healthy adults.

Table 2. Gastric residual rate measured by US, value (%) at various times after the test meal, before and after treatment in children with functional dyspepsia in the three groups

Groups	n	after 30 min		60 min		90 min		120 min	
		Before	After	Before	After	Before	After	Before	After
Group I	40	66.73±11.46	60.85±12.92	48.77±10.76	42.33±9.11*	36.43±9.66	30.10±8.83*	25.03±8.91	19.92±8.81*
Group II	40	64.42±10.30	60.46±8.79	47.38±9.55	44.39±6.66	37.27±8.73	34.18±5.94	24.44±4.74	22.95±4.70
Group III	40	64.12±7.97	58.94±8.13	49.61±6.71	41.63±7.37*	37.62±7.19	28.45±5.69*	25.20±6.91	18.49±4.92*
F value		0.403		4.93		6.40		4.61	
P		>0.05		<0.05		<0.05		<0.05	

F is the comparison of increased values among groups before and after treatment; * shows what is compared with that before treatment has P<0.01.

Table 3. Measured by US average skin thickness, upper arm girth, body fat percentage and BMI before and after treatment in children with functional dyspepsia in the three groups studied.

Groups	n	Average skin thickness (mm)		Upper arm girth (cm)		Body fat percentage		BMI	
		Before	After	Before	After	Before	After	Before	After
Group I	40	5.56±1.17	6.24±1.17*	14.10±1.51	15.90±1.51*	12.15±1.07	14.02±1.11*	14.38±1.4	15.04±1.63
Group II	40	5.20±0.90	5.84±0.84	13.58±1.84	14.37±1.82	12.59±0.83	13.20±0.83	14.47±1.92	15.16±2.09
Group III	40	5.38±1.09	7.25±1.17*	13.84±1.80	15.64±1.70*	12.74±0.93	14.46±0.99*	13.96±1.31	14.85±1.31*
F value		16.80		21.06		46.9		1.082	
P		>0.05		<0.05		<0.05		<0.05	

F is the comparison of increased values in the three groups before and after treatment; * shows what is compared with that before treatment, P<0.01.

Discussion

Hippophae rhamnoides is rich in bioactive components as vitamins, amino acids, organic acids, lipids, flavonoids and 5-HT etc. The results of this study showed that the individuals treated with hippophae rhamnoides (Groups I and III) had much higher levels of LP (inhibiting appetite) and NPY (promoting appetite) in plasma as compared to those who did not take hippophae. This indicates that treatment hippophae rhamnoides dry emulsion may increase the levels of the two appetite regulators, LP and NPY in children with functional dyspepsia, which could be related to the various bioactive components of hippophae rhamnoides. We could find no similar study in the literature available to us.

Children FD has often been a focus of research. Neuropeptide Y and LP have been studied alone or in pairs. Neuropeptide Y and LP are a pair of important brain-gut peptides in the brain appetite center and important factors in regulating gastrointestinal motility. Leptin is a product of leptin gene, and a protein composed of 167 amino acids secreted from a white fat cell. One may regulate his body energy metabolism and body fat by taking more or less LP. Children with FD usually have significantly lower levels of LP and NPY than healthy children [8, 9]. Decrease of fat in the diet of sick children causes decrease of the secretion of LP.

There are many techniques to check the gastrointestinal motility, such as radiology, radionuclide imaging, esophageal manometry, ultrasound, breath test etc. The principle of radionuclide imaging is to blend the radionuclide-labeled drug in ordinary food. Because the process of radioactive nuclide in intragastric movement is completely consistent with that of food movement, we can get the dynamic functional image of the stomach in vitro with gamma camera continuous dynamic views till the time of gastric half emptying-time of the slope of the radioactive curve after computer processing. We can also calculate various factors, such as the GE rate at different times [10]. This method was considered as the gold standard for the determination of GE. Because the nuclear medicine method is convenient and accurate, it is accepted by many physicians for clinical studies.

Kim DY (2004) [11] suggested measuring gastric emptying time with 3-D ultrasonography. Others [12] selected 10 patients with non-ulcer dyspepsia to measure GE time with mixed solid-liquid test meal [13] by using technetium-99m pertechnetate ($^{99m}\text{TcO}_4^-$) applying this imaging and the US method. The gastric liquid meal T1/2 was similar as measured by both methods. Gastric emptying rate and GE time were in a straight line correlation. This correlation indicated that measuring GE time with the single slice of real-time US method and with the nuclear medicine method gave no significant difference between them. This study tested GE by the nuclear medicine technique in adults to indicate that the US method we used was as sensitive as the nuclear medicine method.

In our study 14 healthy adults, volunteers underwent the GE test of mixed liquid-solid food by B US and by the radionuclide technique, and the results showed that both curves were similar as measured at three different times. We note that the radionuclide imaging method is not usually recommended in children due to its low-dose radiation. Furthermore, it is more difficult and expensive.

Leptin can inhibit GE as other researchers [14] observed

that injection of LP into rats' abdomen could delay GE. Others [15] studied how gastric movements worked in rats when they were injected with leptin in the ventromedial hypothalamic area (VMH) and the lateral hypothalamic area (LH). They found that LP inhibited the contraction of migrating motor complex (MMC) phase III. It worked better in VMH than in LH, which indicated that LP could regulate the gastric movement in rats. Leptin is the hormone that initiates MMC phase I activity in empty stomach and any change in LP can cause delay of GE related to the feeling of fullness. In this study LP was increased in subjects treated with hippophae rhamnoides, which was correlated with the significant increase in the average thickness of sebum, upper arm girth and body fat. The increase of LP could inhibit GE. However, the fact children had better gastric mobility after treatment with hippophae may be related to the increased secretion of NPY, though this requires further research.

Neuropeptide Y is a strong appetite stimulant that plays an important role in the taking, storing, consuming and balancing of energy in organisms. Normally, the increase of NPY in the brain promotes appetite, increases the taking of food and also can decrease sympathetic nerves function on brown fat tissue. This can result in producing less heat and increasing the expression of enzymes related to lipid synthesis inside the white fat tissues, thus, increasing stored fat and weight [16].

Neuropeptide Y is closely related to LP. Researchers [17] injected LP into the ventricle of rats, making them eat less, lose weight and also their mRNA and NPY decreased in the arcuate of the hypothalamus area (ARC). Neuropeptide Y is the major intermediary for activating LP, which thus, keeps energy in balance through NPY. Gastrointestinal mobility could have been supported by hippophae per se possibly by the B vitamins it contains, which could promote the synthesis of acetylcholine [18] that stimulates and strengthens gastric mobility, lengthens its rhythmic contraction cycle, increases the amplitude of its contractions and promotes appetite [19]. It has also been suggested that hippophae increases blood flow in rats [20].

Domperidone is a strong dopamine receptor antagonist able to overcome barriers in the stomach. However, it may affect appetite of children with FD. This study showed that in contrast to hippophae rhamnoides, domperidone did not greatly improve children's appetite, and this may be due to the fact that appetite regulators were not secreted.

In conclusion, hippophae rhamnoides can increase the production of LP and of NPY in children with functional dyspepsia. The overall effect of hippophae rhamnoides is improvement of gastric emptying, gastric mobility, gastrointestinal digestive function and promotion of children's growth.

The authors declare that they have no conflicts of interest.

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