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BRIEF ARTICLE

Effect of *Gongronema latifolium* on gastric emptying in healthy dogs

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Abstract

AIM: To investigate sonographically the effect of *Gonogronema latifolium* (*G. latifolium*) on gastric emptying of semi-solid meals in healthy dogs.

METHODS: In a randomized, placebo-controlled experiment, twenty-five clinically healthy dogs were randomly allotted into five groups of five dogs in each group. The placebo group served as the control, and the low, moderate and high dose groups ingested the methanolic leaf extract of *G. latifolium* in capsules at 100 mg/kg, 250 mg/kg and 500 mg/kg, respectively, while the prokinetic group ingested 0.5 mg/kg capsules of metoclopramide. After a 12-h fast, each group ingested its treatment capsules 30 min before the administration of a test meal. Measurements of gastric emptying and blood glucose levels were obtained 30 min before and immediately after the ingestion of the test meal and thereafter every 15 min for 4 h. This was followed by further measurements every 30 min for another 2 h.

RESULTS: The gastric emptying times of the placebo, low dose, moderate dose, high dose and prokinetic dose groups were 127.0 ± 8.2 min, 135.5 ± 3.7 min, 155.5 ± 3.9 min, 198.0 ± 5.3 min and 59.0 ± 2.5 min, respectively. Gastric emptying times of the moderate and high dose groups were significantly slower than in the placebo control group (155.5 ± 3.9 min, 198.0 ± 5.3 min *vs* 127.0 ± 8.2 min, $P = 0.000$). No significant difference in gastric emptying between the low dose and placebo control groups was noted (135.5 ± 3.7 min *vs* 127.0 ± 8.2 min, $P = 0.072$). Gastric emptying of the prokinetic group was significantly faster than that of the control group (59.0 ± 2.5 min *vs* 127.0 ± 8.2 min, $P = 0.000$). The hypoglycaemic effect of *G. latifolium* and gastric emptying were inversely related ($r = -0.95$, $P = 0.000$).

CONCLUSION: *G. latifolium* delays gastric emptying and lowers postprandial blood glucose in healthy dogs. It reduces the postprandial blood glucose by delaying gastric emptying.

Key words: *Gonogronema latifolium*; Gastric emptying; Sonography; Postprandial blood glucose; Semi-solid meals

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INTRODUCTION

Gastric emptying (GE) is the rate with which substances

leave the stomach after ingestion[1]. The process involves the storage of food, mixing with gastric secretions, grinding the solid food into particles of 1-2 mm in diameter, and subsequent delivery of the chyme into the small intestine at a rate designed to optimize digestion and absorption[2]. GE is one of the factors that affect the rate and completeness of intestinal nutrient absorption[3] and is a major determinant of postprandial glycaemic excursions not only in healthy subjects but in type 1 and type 2 diabetic patients[2].

The GE process can be influenced by a variety of physiological, pathological, pharmacological and dietary factors[4]. The effect on GE of *Gongronema latifolium* (*G. latifolium*) (Asclepiadaceae), which is used as a dietary and pharmacological agent for the control of postprandial blood glucose excursions[5,6], has, as far as we know, not been investigated previously. None of the relatively scanty information available on the mechanism(s) of action of *G. latifolium* relates to its effect on GE. We hypothesized that ingestion of a hypoglycaemic agent like *G. latifolium* might accelerate gastric emptying in healthy individuals just as insulin does[7].

In this study we investigated with the use of ultrasonography the effect of the methanolic leaf extract of *G. latifolium* on GE in health, as well as the relationship between its hypoglycaemic effect and GE, using the dog as an animal model. The study further investigated whether the effect of *G. latifolium* on GE in healthy individuals is dose dependent. This study is important as new therapies that aim to change blood glucose by modulating GE are being actively explored and evaluated.

MATERIALS AND METHODS

Plant preparation

Fresh *Gongronema latifolium* leaves were supplied, identified and authenticated by Mr. Ozioko AO, a taxonomist with the Bioresources Development and Conservation Programme Center, Nsukka, South Eastern Nigeria, an International Centre for Enthomedicine and Drug Development (INTERCEDD). A voucher specimen (INTERCEDD/170) was deposited for reference at the centre. The fresh *G. latifolium* leaves were air-dried, pulverized and the extract was prepared according to the previously described method by Ugochukwu and Babady[5]. The 2.2 kg dried powder of *G. latifolium* was extracted with 80% Romil-SA Methanol (MRS Scientific Ltd Essex United Kingdom) and dried in a hot air oven (Gallenkamp, England) at 40 ℃. The filtrates were concentrated at 40 ℃ using a vacuum rotary evaporator and freeze-dried to yield about 146.6 g of green coarse powder. The powder was further pulverized and encapsulated in doses of 100 mg, 250 mg and 500 mg for use in the study.

Animals

Clinically healthy mongrel dogs with no clinical and laboratory evidence of gastrointestinal disease, diabetes, gastroparesis, cardiovascular, pulmonary, renal, and hepatic diseases as ascertained by a veterinary doctor were used in this study. Pregnant female dogs confirmed by palpation and ultrasound were excluded. The dogs were dewormed with 5 mg/kg Levamizole® (Levamisole hydrochloride, Eagle chemical Co. Ltd, N. Korea) one week prior to the GE study. Food was withheld from the dogs for 12 h while water was withheld for 2 h before the study. The age, weight and fasting blood glucose concentration of the dogs did not differ between the control and the treatment groups ($P = 0.7$; $P = 0.2$; $P = 0.7$) (Table 1).

Table 1 Demographic and baseline clinical characteristics of the healthy groups (mean ± SD)

Characteristics	Placebo control	Low dose	Moderate dose	High dose	Prokinetic dose
Age (mo)	6.6 ± 0.7	6.5 ± 1.1	5.9 ± 0.8	6.4 ± 1.1	6.1 ± 0.3
Weight (kg)	6.2 ± 0.7	5.3 ± 0.3	5.6 ± 0.4	5.8 ± 0.7	5.7 ± 0.5
FBG (mmol/L)	4.0 ± 0.6	3.9 ± 0.1	4.0 ± 0.5	3.9 ± 0.2	4.2 ± 0.1

FBG: Fasting blood glucose.

Test meal

The test meal used consisted of 100 g proprietary canned Nestle Cerelac (Maize and Milk infant cereal, Nestle Nigeria plc) food and 150 mL of water. The calories and nutritional components in the 100 g food and 150 mL water are: Calories 1730 KJ (414 kcal); Protein 15 g; Fat 9 g; Carbohydrates 68.2 g; Dietary fibre 2 g; Minerals (ash) 3.3 g; Moisture 2.5 g.

Study design

The study was approved by the University of Nigeria Ethical Committee UNTH Enugu. The guidelines of the National Institutes of Health (NIH) *Principles of Laboratory Animal Care* (NIH Publication No. 86-23, revised 1985) were followed. This clinic-based experimental study was carried out in the University of Nigeria Veterinary Teaching Hospital, Nsukka. A randomized, placebo-controlled experimental design was adopted in this study and the dog was used as an animal model because of its established performance in the assessment of gastrointestinal motility[3] and in many physiological and pharmacological studies[8]. The dogs were randomly allotted into five groups of five dogs in each group. The placebo group served as the control; the low, moderate and high dose groups ingested the *G. latifolium* leaf extract capsules at 100 mg/kg, 250 mg/kg, 500 mg/kg, respectively, while the prokinetic dose group ingested 0.5 mg/kg capsules of metoclopramide (Mederax® 10 mg, Jiangsu Peng YAO Pharmaceutical Inc China). The prokinetic dose group served as prokinetic control. The prokinetic effect of metoclopramide is comparable to the insulin effect on gastrointestinal motility[7].

After a 12-h fast, each group ingested its treatment capsules 30 min before the administration of the test meal. Measurements of GE and blood glucose levels were obtained 30 min before and immediately after the ingestion of the test meal and then every 15 min for 4 h for each dog. Further measurements were made every 30 min for another 2 h. The three doses of *G. latifolium* were

Table 2 Gastric emptying and incremental postprandial blood glucose concentration of the healthy groups (mean ± SD)

Group	GE (min)	AUC of *i*PPBG (mmol/L × min)
Placebo control	127.0 ± 8.2	1028.6 ± 204.2
Low dose	135.5 ± 3.7	938.1 ± 40.0
Moderate dose	155.5 ± 3.9[b]	559.1 ± 101.8[b]
High dose	198.0 ± 5.3[b]	223.5 ± 52.2[b]
Prokinetic dose	59.0 ± 2.5[b]	1426 ± 108.2[b]

[b]$P < 0.0001$ *vs* control group. *i*PPBG: Incremental postprandial blood glucose concentrations; GE: Gastric emptying; AUC: Area under the curve.

introduced to assess its dose-dependent effect. The minimum dose of 100 mg/kg was based on the dose used in mice and rats[5,9]. All the treatment capsules ingested by the dogs were visually identical. The dogs ingested the test meal under natural free-feeding circumstances.

Measurement of gastric emptying

Gastric emptying was measured using an ultrasound technique as described by Chalmers *et al*[10] and McLellan *et al*[11]. The examinations were performed using a veterinary ultrasound machine with a 6-8 MHz microconvex transducer (Medison SA-600 v; 2006; Medison Co., Ltd., South Korea). Each dog was gently restrained while erect and the transducer was placed in a longitudinal orientation on the ventral midline, caudal to the xiphoid. The ultrasound beam was maintained in the sagittal plane and directed cranially until the liver was located and the stomach identified immediately caudal to it. The stomach was observed using real-time imaging, allowing the image to be frozen between peristaltic contractions when it was at a constant, maximal distension. Electronic callipers were used to measure the craniocaudal and ventrodorsal diameters of the antrum between the serosal margins. The antral area was calculated by using the software incorporated in the ultrasound machine to predict the area inside the elliptical shape defined by the craniocaudal and ventrodorsal diameters of the stomach. Three measurements of antral area were taken at each time, and their mean was used for further calculations. Baseline values were subtracted from the measurements made at each subsequent time point and the values expressed as a percentage of maximal antral area. The percentages of the maximal antral areas measured during each test were plotted against time. The gastric half-emptying time with ultrasonography (T50) that correlated significantly with $t_{1/2}$ of carbon 13-labelled octanoic acid breath test in dogs[11] was used to describe the rate of GE. The T50 was defined as the time at which the antral area decreased to 50 percent of its maximal area. T50 was calculated by linear interpolation between two points in the curve.

Measurement of blood glucose

Five millilitres of blood was drawn from each dog's ear at specific time intervals indicated in the study design. Blood glucose was determined by using a portable Accu-chek® Advantage glucometer (Roche Diagnostics GmbH Mannheim Germany). The incremental blood glucose concentrations were computed and plotted against time, and the blood glucose area under the curve (AUC) was calculated from the blood glucose curve.

Statistical analysis

All the data were expressed as mean ± SD. Dunnett's test was used for parametric multiple comparisons between the control and the treatment groups. Analysis of variance linear trend test was used to assess the dose trend. Pearson correlation was used to assess the linear association between the values of two variables. The values were considered to be significant when the P value was less than 0.05. Graphpad prism version 5.03 for windows (Graphpad Software San Diego California United States) and SPSS 15.0 for Windows Evaluation Version (United States) were used for statistical analysis.

RESULTS

Effect of G. latifolium leaf extract on GE

The GE times of the moderate and high dose groups were significantly ($P = 0.000$) slower than in the placebo control, while the GE of the prokinetic group was significantly ($P = 0.000$) faster than in the placebo control group (Table 2). No significant ($P = 0.072$) difference in GE was observed between the low dose and control groups. The effect of *G. latifolium* on GE was also significantly ($P = 0.000$) dose dependent (Figure 1A).

Effect of G. latifolium leaf extract on postprandial blood glucose concentration

The AUC values of incremental postprandial blood glucose concentrations (*i*PPBG) of the moderate and high dose groups were significantly ($P = 0.000$) smaller than in the placebo control, while the AUC of *i*PPBG of the prokinetic group was significantly ($P = 0.000$) larger than that of the placebo control (Table 2). No significant ($P = 0.442$) difference in AUC of *i*PPBG was observed between the low dose and control groups. The effect of *G. latifolium* on AUC of *i*PPBG of healthy dogs was significantly ($P = 0.000$) dose-dependent (Figure 1B).

The hypoglycaemic effect of G. latifolium and GE

The hypoglycaemic effect of *G. latifolium* and GE were inversely related ($r^2 = 0.95$) (Figure 2).

DISCUSSION

The gastric emptying process can be influenced by a variety of physiological, pathological, pharmacological and dietary factors[4] but the effect on GE of *G. latifolium*, which is used as a dietary and pharmacological agent for the control of postprandial blood glucose excursions[5,6], has, as far as we know, not been investigated in animals/humans previously. In this study, we demonstrate that the ingestion of the methanolic extract of *G. latifolium* delayed the gastric emptying of a semi-solid meal dose-dependently.

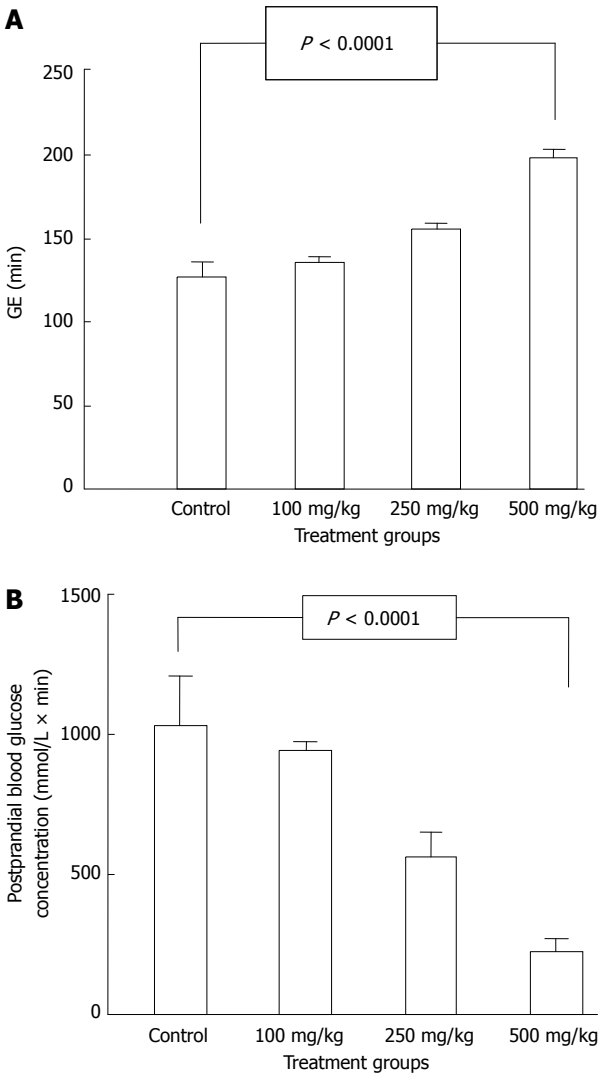

Figure 1 Dose-dependent effect of *Gongronema latifolium* on gastric emptying and postprandial incremental blood glucose concentrations. A: Dose-dependent effect of *Gongronema latifolium* (*G. latifolium*) on gastric emptying; B: Dose-dependent effect of *G. latifolium* on postprandial incremental blood glucose concentrations. GE: Gastric emptying.

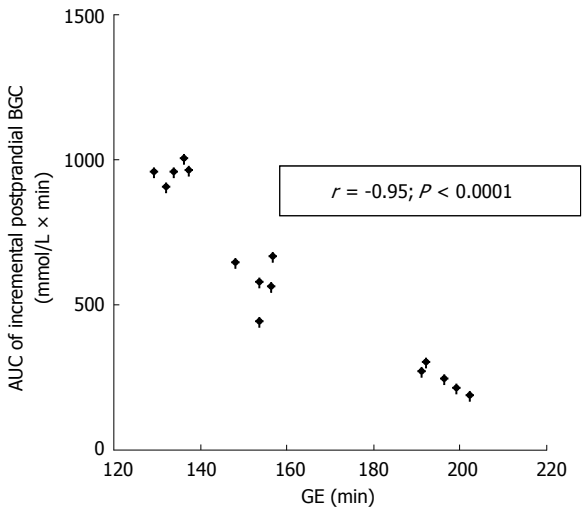

Figure 2 Relationship between the hypoglycaemic and gastric emptying effects of *Gongronema latifolium*. GE: Gastric emptying; AUC: Area under the curve; BGC: Blood glucose concentration.

The effect is similar to that of *Trigonella foenum-graecum*[12] and that of *Gymnema sylvestre*[13,14] which belong to the same Asclepiadaceae plant family as *G. latifolium*[15,16], but opposite to that of metoclopramide as noted in this study.

The presence of saponins in *G. latifolium*[17] and *G. sylvestre*[13,14] may be responsible for the similarity in their GE effects. Saponins significantly and dose-dependently delay gastric emptying[12-14,18]. GE is linearly associated with changes in the antral area[11,19,20]; therefore, our data suggest that *G. latifolium* causes less rapid reductions in antral areas and an increase in antral areas. This agrees with works which associated substances that slow GE with a relative increase in the content of the distal stomach[21,22] and inhibition of antral motility[23]. The more rapid reductions in antral areas and a decrease in antral areas observed with metoclopramide in this study are because it improves GE by increasing amplitude and frequency of antral contractions[4]. Therefore, the observed difference between the GE effect of metoclopramide and that of *G. latifolium* in this study may be due to their different mechanisms of action.

The mechanisms underlying the delayed effects of *G. latifolium* on GE were not evaluated in this study, but we speculate that the effect may be mediated by vagal mechanisms[21,24-26] and/or the release of gastrointestinal hormones[27]. *G. latifolium* may have elicited a gastrointestinal motor and/or sensory function that caused antral distension and subsequently suppressed antral contractions to result in a slower rate of antral delivery of the ingested semi-solid meals. The demonstration of the potential of *G. latifolium* to slow the rate of antral delivery of the ingested semi-solid meals from the stomach into the small intestine by this study is very important as new therapies that aim to change blood glucose by modulating GE are being actively explored and evaluated. The effect has hitherto not been demonstrated in animals or humans.

This study also demonstrates that there is an inverse relationship between the GE effect of *G. latifolium* and blood glucose concentration. It agrees with the fact that the pharmacologic acceleration of GE results in higher postprandial glucose concentrations, while delaying GE results in lower postprandial glucose concentrations after a physiologic meal[28]. Although the demonstration of a correlation does not establish causation, this finding suggests that *G. latifolium* when ingested with a meal may, through the mechanism of delayed GE, slow digestion and prolong the postprandial absorption of food, with a resultant improvement or reduction in postprandial blood glucose concentrations after a semi-solid meal. The rates of meal-derived glucose appearance in the systemic circulation are determined mainly by GE[29,30]. A previous work indicates that saponins with hypoglycaemic activity also inhibited GE while other saponins that have no hypoglycaemic activity did not affect GE[31]. Thus, saponins in *G. latifolium* may be responsible for the correlation between GE and its hypoglycaemic effects in healthy dogs. Some authors have proposed that saponin compounds act as hypogly-

caemic agents by delaying the transfer of glucose from the stomach to the small intestine, the main site of glucose absorption, and by inhibiting the glucose transport at the site of intestinal brush border membranes[32,33].

In this study, 100 mg/kg of *G. latifolium* did not significantly slow GE, probably due to low dose-response effect. The 100 mg/kg dose of *G. latifolium* might be below the threshold required to cause a significant delay on GE. Our findings that *G. latifolium* in a dose-dependent manner affects both the rate and extent of carbohydrate absorption by slowing the transfer of food from the stomach into the small intestine and thereby reducing or delaying exposure of nutrient to small bowel mucosa are clinically relevant with regard to improving postprandial blood glucose and triglycerides and consequently lowering the risk of chronic disease. Distension of the stomach is one factor that promotes the feeling of satiety[34]. Therefore, *G. latifolium* may, through the mechanism of delayed GE, play an important role in the regulation of appetite and energy intake. Finally, since interventions directed at modulating upper gastrointestinal motor and absorptive functions have a major effect on postprandial blood glucose excursion and are likely soon to enter the mainstream of therapy for diabetes[2,35], *G. latifolium* may be relevant in the current treatment and management of diabetes. Alternatively hyperglycaemia can cause a decrease in GE rate[36,37]. This factor is unlikely to have influenced the result of this study much since there was no statistically significant difference between the preprandial blood glucose concentrations in the subgroups when compared with the placebo control.

The neural, humoral and cellular mechanisms by which *G. latifolium* affects GE were not investigated, therefore further studies are required to elucidate them. Although the dog is an animal model for the study of human GE in many physiological and pharmacological studies[8] and an established model for the assessment of gastrointestinal motility[3], the pattern of findings may not be exactly the same in humans as demonstrated in this study.

Gongronema latifolium delays GE and lowers postprandial blood glucose in healthy dogs. It reduces the postprandial blood glucose by delaying GE. The effect has also been noted to be dose-dependent. Therefore it can play an important role in the current dietary and pharmacological approaches in the prevention, treatment and management of metabolic diseases like diabetes.

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COMMENTS

Background

Gastric emptying is a major determinant of postprandial glycaemic excursions not only in healthy subjects but in diabetic patients. Interventions directed at modulating gastric emptying have a major effect on postprandial blood glucose excursion. *Gongronema latifolium* (*G. latifolium*) (Asclepiadaceae) is currently used as a dietary and pharmacological agent for the control of postprandial blood glucose excursions, but its effect on gastric emptying has, as far as we know, not been reported.

Research frontiers

Previous studies have demonstrated the hypoglycaemic effect of *G. latifolium*, but none of the relatively scanty information available on its mechanism(s) of action relates to its effect on gastric emptying. In this study, the authors demonstrated that the gastric emptying delaying effect of *G. latifolium* could be one of the pathways for reducing the postprandial blood glucose level.

Innovations and breakthroughs

New therapies that aim to change blood glucose by modulating gastric emptying are being actively explored and evaluated. This is believed to be the first study to explore the effect of *G. latifolium* on gastric emptying in health as well as the relationship between its hypoglycaemic effect and gastric emptying.

Applications

The findings that *G. latifolium* in a dose-dependent manner slows the rate of antral delivery of the ingested semi-solid meals from the stomach into the small intestine may be clinically relevant in the management approach of postprandial blood glucose and triglycerides and consequently in diabetics.

Terminology

Gastric emptying is the rate with which substances leave the stomach after ingestion. The process involves the storage of food, mixing with gastric secretions, grinding the solid food into particles of 1-2 mm in diameter, and subsequent delivery of the chyme into the small intestine at a rate designed to optimize digestion and absorption. *G. latifolium* (Asclepiadaceae) is an edible tropical rainforest plant that is widely used in folk medicine.

Peer review

The authors present data on the effects of various doses of the methanolic leaf extract of *G. latiifolium* on gastric emptying and postprandial plasma glucose levels in a dog animal model. Gastric emptying was measured by ultrasonography. The data demonstrate dose related effects on inhibiting gastric emptying and plasma glucose.

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