http://dx.doi.org/10.4314/ajtcam.v10i5.17

GONGRONEMA LATIFOLIUM DELAYS GASTRIC EMPTYING OF SEMI-SOLID MEALS IN DIABETIC DOGS

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Abstract

The aim of the study was to investigate sonographically the effect of *Gongronema latifolium* on gastric emptying of semi-solid meals in diabetic dogs. Twenty-five alloxan-induced diabetic dogs were randomly allotted into five groups of five dogs each in a randomised placebo-controlled study. These are placebo, prokinetic dose, low dose, moderate dose and high dose groups. The placebo group served as the control. The low, moderate and high dose groups ingested methanolic leaf extract of *G. latifolium* at 100 mg/kg, 250 mg/kg, 500 mg/kg respectively, while the prokinetic group ingested 0.5 mg/kg of metoclopramide. After a 12-hour fast, each group ingested its treatment capsules 30 minutes before the administration of test meal. Measurements of gastric emptying and blood glucose levels were obtained from each dog 30 minutes before and immediately after the ingestion of a test meal, every 15 minutes for another 4 hours and then every 30 minutes for further 2 hours. Gastric emptying of the moderate and high dose groups were 227.8 ± 9.9 min and 261.3 ± 19.3 min respectively and significantly ($p < 0.0001$) slower than the placebo control group of 143.0 ±17.8 min. The gastric emptying of the low dose group (169.8 ± 3.8) and control group did not differ significantly ($p > 0.05$). A strong inverse relationship between gastric emptying and the incremental blood glucose levels was noted in the diabetic dogs after the ingestion of *Gongronema latifolium* ($r = - 0.90$; $p < 0.0001$). *Gonogronema latifolium* delayed gastric emptying in diabetic dogs.

Keywords: Diabetes, Gastric emptying, *Gongronema latifolium*, Sonography, Postprandial blood glucose, Antral area

Introduction

Diabetes Mellitus (DM) is a group of metabolic diseases characterised by chronic hyperglycaemia resulting from defects in insulin secretion, insulin action or both (WHO, 1999). It is one of the five leading causes of death in the world and likely the fastest growing metabolic disease (Ugochukwu and Babady, 2003; Ugochukwu et al., 2003). DM is a serious public health problem which is expected to increase considerably over the coming decades (IDF, 2000) due to population growth, aging, urbanisation and increasing prevalence of obesity and physical inactivity (Wild et al., 2004). Complications like retinopathy, nephropathy, neuropathy, cardiovascular disease and the presence of endothelial dysfunction have been reported in diabetes (ADA, 2003; Esper et al., 2006; Ferris et al., 1999; Ritz and Orth, 1999). But these complications are far less common and less severe in people who have well-controlled blood sugar levels (DCCT, 2005; Nathan et al., 2005).

Achieving a well-controlled blood sugar level in diabetics can be difficult (Aronoff et al., 2004; Darwiche et al., 2001; Zia et al., 2001) because most of the therapeutic agents are ineffective (Khan et al., 2012; Tiwari and Rao, 2002) and do not perfectly address many of the abnormalities and/or deficiencies of diabetes (Aronoff et al., 2004). Thus, there is a global rise in diabetes-related mortality, although several drugs for diabetes exist today (Olefsky, 2001; Pandey et al., 2011; Tiwari and Rao, 2002). The limitation of currently available antidiabetic agents and the pandemic form of the disease have encouraged the use of alternative therapy (Pandey et al., 2011). Medicinal plant products being a great source of biological constituents and effective against diabetes (Khan et al., 2012) are now an attractive alternative to currently used diabetic drugs (Hu et al., 2011; Pandey et al., 2011). They are easily available, cheap, relatively safe (Akah et al., 2011) and also part of our daily diet (Awobajo et al., 2013). *Gongronema latifolium* (Asclepiadaceae) is an example of medicinal herbs with such potentials.

G. latifolium is a perennial crop commonly called “madumaro or arokeke” and “utazi” in the western part and south eastern part of Nigeria respectively (Etim et al., 2008). It is a wild climber widely distributed in the southeastern states of Nigeria (Essien et al., 2007). Studies showed that it contains essential oils, saponins and pregnane (Morebise and Fafunso, 1998; Morebise et al., 2002). Its anti-oxidant, antihypercholestrolemic, antilipidemic and antihyperglycaemic effects (Ugochukwu and Babady, 2003; Ugochukwu et al., 2003) have been reported also. *G. latifolium is* often used to treat and/or prevent diabetes (Akah et al., 2011; Ugochukwu et al., 2003).

Gastric emptying (GE) is a major determinant of postprandial glycaemic excursions in type 1 and type 2 diabetic patients (Horowitz et al., 2002). Even small changes in GE may have a substantial effect on the magnitude and timing of postprandial increases in blood glucose and insulin (Karamanlis et al., 2007; Rayner et al., 2001). Interventions that reduce

http://dx.doi.org/10.4314/ajtcam.v10i5.17

hyperglycaemia by modulating GE have the potential to become mainstream therapies in the treatment of diabetes (Horowitz et al., 2002; Rayner et al., 2001). But previous studies that evaluated the effects of *G. latifolium* on blood glucose concentrations in diabetes (Akah et al., 2011; Ogundipe et al., 2003; Ugochukwu and Babady, 2002; Ugochukwu and Babady, 2003; Ugochukwu et al., 2003) did not evaluate its effect on GE. Since GE can be influenced by a variety of pharmacological and dietary factors (Schmitz and Neiger, 2009), the potential effect of *G. latifolium* on GE could be beneficial in the current approach to diabetes treatment. Most drugs including insulin used to achieve glucose homeostasis in diabetes are agents that accelerate GE (Matsuda et al., 1999). Therefore, we hypothesised that ingestion of a hypoglycaemic agent like *G. latifolium* would accelerate GE in the diabetic.

In this study we therefore investigated with the use of ultrasonography the effect of the methanolic leaf extract of *G. latifolium* on GE in diabetics as well as the relationship between its hypoglycaemic effect and GE using dog as the animal model. The study further investigated if the effect of *G. latifolium* on GE in diabetics is dose-dependent. This study is important because it will provide insights into the effects of *G. latifolium* on GE and it may possibly stimulate investigations that will determine whether *G. latifolium* has the potential to enter the mainstream of therapy for diabetes.

Materials and Methods

Plant preparation

A taxonomist with the Bioresources Development and Conservation Programme, Nsukka, South Eastern Nigeria, Mr. A.O. Ozioko supplied, identified and authenticated the fresh *Gongronema latifolium* leaves. A voucher number INTERCEDD/170 was deposited at the centre which is an International Centre for Enthomedicine and Drug Development. The methanolic *G. latifolium* leaf extract was prepared as previously described (Ogbu et al, 2013). The dried powders of *G. latifolium* were extracted with 80 % Romsil –SA Methanol (MRS Scientific Ltd Essex United kingdom) for 48 hours and the mixtures were filtered with Whatman No.1 filter paper (Schleicher & Schuell, England).The methanolic extract was dried in a hot air oven (Gallenkamp, England) at 40^{o}C for 96 hours. Extract was concentrated at 40^{o}C using a vacuum rotary evaporator and freeze-dried. The resultant coarse power was pulverised and encapsulated in doses of 100 mg, 250 mg and 500 mg.

Animals

Healthy looking male and female Mongrel dogs were purchased from local Orba market, Nsukka. Upon arrival, the dogs were taken to the University of Nigeria Nsukka (UNN) Veterinary Teaching Hospital for clinical/laboratory examination to establish their health status. The dogs were housed in pairs and in cages in the animal house of the Department of Medicine, Veterinary Teaching Hospital Nsukka under controlled environmental conditions of temperature (25 - 28 ^{O}C) and relative humidity (70-80%) and a 12 hour lights/day cycle. Prior to the arrival of the dogs the housing unit was cleaned and disinfected. The dogs were allowed to acclimatise for at least 14 days while being maintained on 300g of a regular commercial dog food Ol! Roy Complete Nutrition twice a day. The guaranteed analysis of the nutritional components of the dog food has been reported earlier (Ogbu et al., 2013). Drinking water was provided ad libitum. The dog house was cleaned regularly; the eating plates and drinking bowl were washed and sun-dried every day.

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 (IUA) 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 hours while water was withheld for 2 hours before the study. The age, pre-diabetic weight, post-diabetic weight, pre-diabetic and post-diabetic fasting blood glucose concentrations of the dogs did not differ (P = 0.92, 0.99, 0.97, 0.89, 0.15) between the placebo control and the treatment groups (Table 1).

Table 1: Demographic and clinical characteristics of the alloxan induced diabetic dogs (Mean ± SD)

Group	Age (months)	Pre-diabetic Weight (Kg)	Post-diabetic Weight (Kg)	Pre-diabetic FBG (mmol/L)	Post-diabetic FBG (mmol/L)
Placebo control	5.7 ± 0.5	3.8 ± 1.1	3.4 ± 1.2	5.3 ± 1.0	15.4 ± 0.5
Low dose	5.4 ± 0.5	3.8 ± 1.0	3.6 ± 1.1	5.5 ± 1.5	14.5 ± 2.0
Moderate dose	5.6 ± 0.9	3.9 ± 0.3	3.7 ± 0.5	5.3 ± 0.5	13.9 ± 1.3
High dose	5.7 ± 0.5	3.9 ± 0.7	3.8 ± 0.8	5.5 ± 0.9	15.1 ± 0.8
Prokinetic dose	5.7 ± 0.6	3.8 ± 1.3	3.5 ± 1.2	4.9 ± 0.4	13.6 ± 1.1
Mean	5.6 ± 0.5	3.8 ± 0.9	3.6 ± 0.9***	5.3 ± 0.9	14.5 ± 1.5***

***p< 0.0001 vs pre-diabetic

Test meal

The test meal which consisted of 100 g proprietary canned Nestle Cerelac (Maize & Milk infant cereal, Nestle Nigeria Plc.) food and 150 ml of water, its calories and nutritional components have been reported previously (Ogbu et al., 2013).

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Induction of diabetes

Diabetes mellitus was induced in dogs used for the study by intravenous (IV) injection of alloxan monohydrate (Sigma, USA) at a dose of 100 mg/kg (Jelodar et al., 2007; Kim et al., 2006). The dogs were fasted for 24 hours prior to the diabetes induction (Kim et al., 2006). Fresh solution of alloxan monohydrate for diabetes mellitus induction was prepared just prior to injection (Anderson et al., 1993; Kim et al., 2006). The dogs were each given a single intravenous injection of 100 mg/kg (100 mg/ml) via the cephalic vein in the foreleg.

Diabetes was confirmed at one week after the alloxan injection by the presence of fasting blood glucose level of above 11.10 mmol/L (Jelodar et al., 2007). Polyuria and polydipsia, the clinical signs of diabetes, were also noted. Only the dogs with induced diabetes mellitus were enlisted into the study. Eighty percent (80 %) of the dogs were male. The post-diabetic weight and post-diabetic fasting blood glucose concentration mmol/L differed significantly ($p < 0.0001$) with their prediabetic values respectively (Table 1).

Study design

The study design was approved by the University of Nigeria Ethical Committee UNTH Enugu. The study protocol conforms to the provisions of the Declaration of Helsinki in 1995 (as revised in Edinburgh 2000). The guidelines of the National Institutes of Health (NIH) *Principles of Laboratory Animal Care* (NIH Publication No. 86-23, revised 1985) were strictly followed. This clinic based study was carried out in the University of Nigeria Veterinary Teaching Hospital, Nsukka. A randomised placebo-controlled experimental design was adopted in this study. The design and the rationales for using the design have been discussed in detail previously (Ogbu et al, 2013). The dogs were randomly allotted into five groups of five dogs in each group. The placebo group served as the baseline control while prokinetic dose group served as prokinetic control. The low, moderate, high dose groups ingested capsules containing the *G. latifolium* leaf extract 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 effect of metoclopramide is comparable to insulin effect on gastrointestinal motility (Reddy et al., 2006). After a 12-hour fast, each group ingested its intervention capsules 30 minutes before the administration of the test meal. Measurements of GE and blood glucose levels were obtained 30 minutes before and immediately after the ingestion of the test meal and then every 15 minutes for 4 hours for each dog. Further measurements were made every 30 minutes for another 2 hours. The three doses of *G. latifolium* were introduced based on previous study (Ogbu et al, 2013). 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 examinations were performed as previously described (Ogbu et al., 2013) using a veterinary ultrasound machine with a 6-8 MHz microconvex transducer (Medison SA-600v; 2006; Medison Co., Ltd Korea). Each dog was gently restrained while erect and the transducer 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 used for further calculations. Baseline values were subtracted from each measurement and the values expressed as a percentage of maximal antral area. The percentages of the maximal antral areas obtained for each 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 (McLellan et. al., 2004) 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

Blood glucose concentrations were determined as previously described (Ogbu et al., 2013). 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 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. Paired t-test used for comparison of two groups and Pearson correlation was used to assess the linear association between the values of two variables. ANOVA linear trend test was used to assess the dose trend.

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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 Califonia USA) and SPSS 15.0 for Windows Evaluation Version (USA) were used for statistical analysis.

Results

Effect of *G. latifolium* leaf extract on GE

The GE of moderate and high dose groups were significantly ($p < 0.0001$) slower than control group. The GE of prokinetic group was significantly ($p < 0.001$) faster than the control group. No significant ($p > 0.05$) difference in gastric emptying was observed between the low dose and control groups (Table 2). The effect of *G. latifolium* on GE of diabetic dogs was significantly ($p < 0.0001$) dose dependent.

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

The AUC of incremental postprandial blood glucose concentration (AUC of iPPBG**)** of moderate and high dose groups were significantly ($p < 0.0001$) smaller than the control group, while the GE of the prokinetic group was significantly ($p < 0.0001$) larger than control group. There was no significant ($p > 0.05$) difference in the AUC of incremental postprandial blood glucose concentration between the low dose and control groups (Table 2). The effect of *G. latifolium* on AUC of incremental blood glucose concentration of the diabetic dogs was significantly dose-dependent ($p < 0.0001$).

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

Group	Extract dose (mg/kg)	Gastric emptying (mins)	AUC of incremental postprandial Blood glucose (mmol/L x mins)
Placebo control	-	143 ± 15	3453 ± 218
Low dose	100	161 ± 6	3357 ± 539
Moderate dose	250	228 ± 9***	1905 ± 434***
High dose	500	261 ± 17***	385 ± 298***
Prokinetic dose	-	111 ± 15**	6485 ± 322***

$^{**}p < 0.001$ *vs* placebo control group. $^{***}p < 0.0001$ *vs* placebo control group.

Hypoglycaemic effect of *G. latifolium and GE*

The effects of *G. latifolium* on the AUC of incremental postprandial blood glucose concentration (AUC of iPPBG**)** and GE were inversely related in the diabetic dogs (Figure 1).

Discussion

It is fundamentally important to understand the relationship between the glycaemic response to carbohydrate and GE (Jones et al. 1996) with a hypoglycaemic agent like *G. latifolium* in the treatment of diabetes mellitus. None of the available information on the hypoglycaemic mechanisms of *G. latifolium* relate to upper gastrointestinal motor function like GE. In this study we investigated with the use of ultrasonography the effect of the methanolic leaf extract of *G. latifolium* on GE in diabetics as well as the relationship between its hypoglycaemic effect and GE using dog as animal model. Whereas the hypoglycemic effect of *G. latifolium* as demonstrated in this study was documented previously (Ogundipe et al.,2003; Ugochukwu and Babady, 2003; Ugochukwu et al., 2003), we have demonstrated for the first time that ingestion of the methanolic leaf extract of *G. latifolium* delays the GE of a semi-solid meal in diabetes dose-dependently. This finding was opposite to our study hypothesis. In addition, we observed that the *G. latifolium* dose-dependently induced delay in GE is associated with a reduction in postprandial blood glucose concentrations.

In diabetes mellitus there is a reduced capacity of the β-cells of the pancreas to release insulin whether the cells are destroyed or intact (Panneerselvam and Govindaswamy, 2002). Our experimental animals were treated with alloxan that resulted in fasting blood glucose level of above 11.10 mmol/l, polyuria, polydipsia and decreased weight in the dogs. Taking into account that alloxan is an agent that selectively destroys the β-cells (Balogh et al., 2008) and decreases the endogenous insulin secretion (Yamamoto et al., 1981), our findings indicate that *G. latifolium* through mechanism of delayed GE may have resulted in gradual release of nutrients for absorption to match the lower levels of insulin and prevent the accumulation of excess glucose. Gastric emptying is one of the factors that affect the rate and completeness of intestinal nutrient absorption (Xu et al., 2005). "Therefore, it seems reasonable to speculate that *G. latifolium* attenuates blood glucose excursions partly through delayed GE, thus delaying the transfer of glucose from the stomach to the small intestine. The main site of glucose absorption (Francis et al.,2002; Tiwari and Rao, 2002) may be an additional mechanism hitherto unknown through which *G. latifolium* exerts its hypoglycaemic effect in diabetics."

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It is also likely that the dominant effect of *G. latifolium* on postprandial glycaemia could be mediated by the delaying of GE since GE is a major determinant of postprandial glycaemic excursions in type 1 and type 2 diabetic patients (Horowitz et al., 2002; Rayner et al., 2001).

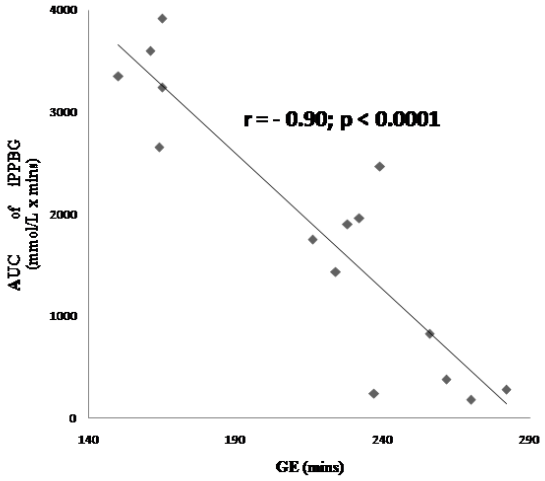

Figure 1: Relationship between the hypoglycaemic effect of *G. latifolium* and gastric emptying in diabetic dogs treated with *G. latifolium*. n =15

Gastric emptying can be influenced by a variety of pharmacological and dietary factors (Schmitz and Neiger, 2009). Thus, therapies aimed at regulating GE are being actively explored and applied clinically for the management of diabetics. To date, the underlining mechanisms of *G. latifolium* hypoglycaemic action is not fully understood. This study provides an insight into the effects of *G. latifolium* on GE, which in line with the current treatment and management of diabetes, is relevant to the understanding of its hypoglycaemic action. Since modulation of GE by dietary and pharmacological means to minimise postprandial glucose excursions represents a new approach to improving postprandial glycaemic control (Horowitz et al., 2002; Rayner et al., 2001), this study has demonstrated that *G. latifolium* has potential to be effective in the current treatment and management of diabetes.

The study findings are similar to previous findings in healthy dogs (Ogbu et al., 2013) but are contrary to that of treatment with insulin and other hypoglycaemic agents including metroclopramide, in patients and animals with diabetes (Matsuda et al., 1999; Robain et al., 1995; Schmitz and Neiger, 2009). Metroclopramide increases amplitude and frequency of antral contractions (Schmitz and Neiger, 2009) to improve gastric emptying (Robain et al., 1995) while insulin has been reported to accelerate the GE through hypoglycaemic effect (Reddy et al., 2006).

The male sex bias of the dogs used for the diabetic study is unlikely to influence the result, since previous works in dogs have not noticed gender effect on GE (Allan,et al.,1996; Yam et al., 2004). Hyperglycaemia can cause a decrease in gastric emptying rate in diabetic individuals (Koch, 1999; Vinik et al., 1999) and could have affected this study. This factor is unlikely to have influenced the result much since there was no statistical significant difference ($p > 0.05$) between the preprandial blood glucose concentrations in the treatment groups and placebo control.

The rapid spread of type 2 diabetes mellitus is a growing burden on healthcare worldwide (Heine et al., 2006). Drugs that reduce postprandial hyperglycaemia by suppressing the absorption of carbohydrate are effective in the type 2 diabetes mellitus prevention and treatment (Tiwari and Rao, 2002). In type 2 diabetes mellitus, characterised by diminished and delayed endogenous insulin release (Rayner and Horowitz, 2005), the use of G. *latifolium* will actually improve glycaemic control. Thus, the findings of this study have implications for the prevention and clinical management of type 2 diabetes mellitus.

Metoclopramide accelerated gastric emptying in diabetic dogs, but its prokinetic effect appeared moderate ($p < 0.001$) compared to that of placebo control in this study. This may be due to the high incremental postprandial blood glucose concentration of the diabetic prokinetic dose group compared to the placebo control group. Hyperglycaemia has been shown to attenuate the prokinetic effect of intravenous erythromycin on GE in diabetic patients (Petrakis et al., 1999). It is likely that the action of other prokinetic drugs is impaired by hyperglycaemia (Rayner et al., 2001) including metoclopramide (Chapman et al., 2009). Therefore, the frequency of antral contractions caused by metoclopramide (Schmitz and Neiger, 2009), may have been attenuated mildly by hyperglycaemia.

http://dx.doi.org/10.4314/ajtcam.v10i5.17

The effect of *G. latifolium* on DM patients with gastroparesis was not studied. The pattern of findings may not be exactly the same as demonstrated in this study. Whether *G. latifolium* treated DM patients with gastroparesis will present adverse hypoglycaemic episodes need to be ascertained.

In conclusion, *G. latifolium* delays gastric emptying in diabetic dogs. *G. latifolium* improves glucose homeostasis in diabetic dogs by mechanisms that may be involved with delayed GE.

Acknowledgement

We would like to thank Dr K.O. Anya of University of Nigeria Veterinary Teaching Hospital, Nsukka for the assistance in taking care of the experimental dogs.

The authors declare that they have no conflict of interest.

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