Inhibitory Effects of *Glyphaea brevis* Spreng. (Monach.) on Pancreatic α-Amylase Activity: Impact on Postprandial Blood Glucose and Weight Control in Rats

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ABSTRACT

This study investigates the possible utilisation of *Glyphaea brevis* Spreng. (Monach.), a Tiliaceae family member, as a novel source of α-amylase inhibitors or "starch blockers". Aqueous (AE) and hydroalcoholic (HAE) extracts were prepared from the leaves and their effects on pancreatic α-amylase activity were assessed *in vitro* using starch and 4,6-ethylidene-(G7)-*p*-nitrophenyl-(G1)-α,D-maltoheptaoside (ethylidene-G_7PNP) as substrates. Both extracts were administered at doses of 250 and 500 mg/kg to male albino Wistar rats over a 4-week period and their effects on oral starch tolerance (OST), weight gain and faecal output were evaluated and compared to a control group. Both extracts showed an inhibitory effect on α-amylase *in vitro* (AE: starch: 43.09%; ethylidene-G_7PNP: 94.59%; HAE: starch: 52.05%; ethylidene-G_7PNP: 98.31%). They also improved OST, increased fresh and dry faecal weight ($p<0.05$), and significantly reduced weight gain ($p<0.001$) in rats over the experimental period. All these effects appeared to be dose-dependent for each extract. These results suggest that *G. brevis* can constitute a source of α-amylase inhibitors reducing dietary starch digestibility, glucose bioavailability and, therefore, endogenous lipogenesis. This finding may find applications in the areas of human obesity management and weight loss.

INTRODUCTION

Obesity has become a major public health concern as it nowadays affects about 400 million adults around the world. Also among children and adolescents the prevalence of obesity is increasing (Ogden *et al.* 2006; Bovet *et al.* 2008). The World Health Organization has classified this as a global epidemic of obesity (globesity) and emphasised that it is not restricted to industrialised countries (World Health Organization 1997). It is anticipated that by the year 2015, 2.3 billion adults will be overweight and 700 million will suffer from obesity (World Health Organization 2006). The medicare cost burden of obesity is considerable, and increasing along with the epidemic. As a matter of fact, for American young adults in the obese I class, this cost range from $5,340 for black women to $21,550 for white women. In the obese II/III class, black men have the lowest lifetime cost estimates, $14,580, and white women again have the highest lifetime cost estimates, $29,460. For men, the costs of obese II/III are similar to those of obese I (Finkelstein *et al.* 2008). Unfortunately, current strategies for prevention and treatment have failed to reverse the progression of obesity and therefore, a search for modifiable causes remains imperative.

The development of obesity is determined by both genetic and environmental factors. The increase of carbohydrates in Western diets has a considerable input on the onset of obesity since carbohydrates (glucose and fructose) are substrates for the hepatic synthesis of body fat, also known as lipogenesis (Hudgins *et al.* 2000; Foufelle and Ferré 2004). Therefore, inhibition of lipogenesis has emerged as an opportune strategy in the pharmacological treatment of obesity and has been achieved through the reduction of glucose availability in the bloodstream. Dietary fibers, especially of soluble type, have been effective in the reduction of postprandial glycemia through the inhibition of dietary glucose absorption (Jenkins *et al* 1978; Dakam *et al.* 2007). Alternately, inhibition of the release of glucose from starchy foods through α-amylase inhibition has been the focus of several human and animal studies (Garrow *et al.* 1983; Umoren and Kies 1992; Thom 2000). This inhibition has been reported in some cases to be of competitive type through the binding of inhibiting molecules to substrate or to α-amylase itself, thus reducing starch digestion (Alam *et al.* 2001). Interestingly, most of the molecules effective in α-amylase inhibition, like phenolics, peptides and glycosides were derived from plant products like beans (Marshall and Lauda 1975), olive leaves (Komaki *et al.* 2003), wheat (Choudhury *et al.* 1996), some fruits (McDougall *et al.* 2005), pears, lentils and coca (Queseda *et al.* 1995). These facts suggest the opportunity to investigate further plant sources of α-amylase inhibitors in order to obtain a low-cost and easily-available treatment of obesity.

Glyphaea brevis Spreng. (Monach.), of the Tiliaceae family, is widely distributed in Africa and South America. It is valued as a vegetable and as a medicinal plant. Many of its pharmacological uses and activities have been reported. For instance, it is used in Cameroon for the treatment of palpitations, hepatitis and poisoning (Adjanohoun *et al.* 1996). Some authors reported the use of *G. brevis* in Nigeria in the relief of sleepiness, bacterial infections, convulsions, sexual impotency and some age-related brain disorders (Bhat *et al.* 1990; Fatumbi 1995; Ogbonnia *et al.* 2003). In the Democratic Republic of Congo, the plant is used to treat cough, heart and tooth diseases (Terashima and Ichikawa 2003). Although *G. brevis* appears to be a useful medicinal plant, no study has been done so far on its possibility as a source of α-amylase inhibitors ("starch blockers") that may find applications in the areas of human obesity and weight loss.

Original Research Paper

The aim of this study is therefore to assess the possible inhibitory effects of extracts of *G. brevis* on the activity of α-amylase *in vitro*, using different methods, and *in vivo* in rats. We also evaluate the relationship between these inhibitory effects, weight gain and faecal output.

MATERIALS AND METHODS

Experimental animals

Male Wistar rats bred in our laboratory, 24-weeks old and weighing 240-270 g, were maintained in cages under controlled conditions (25°C, 12: 12-h dark/light cycle) with free access to food and water. Investigations using experimental animals were conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the United States guidelines (United States National Institutes for Health publication no. 85-23, revised in 1985).

Harvesting and processing

Green adult leaves from the aerial part of several 6 m-high plants of *G. brevis* were collected between March and May, 2007 in the city of Douala, Cameroon. The taxonomy was confirmed by Mr. Nana, Cameroon National Herbarium, Yaounde, Cameroon (voucher specimen no.10781/SRF/Cam). The leaves were dried under shade at 40°C and ground into a fine powder using a FFC-37 mill (Agro-Mac, Douala, Cameroon).

Aqueous extraction

150 g of dry leaf powder were introduced in 2 L of distilled water and the mixture was boiled for 15 min. After cooling, this was filtered and the resulting filtrate was smoothly evaporated at 50°C. This process led to a powder referred as aqueous extract (AE) that was kept in a sealed flask at 2°C. The yield of the extraction was 18.8% (w/w).

Hydroethanolic extraction

150 g of dry leaf powder were introduced in 2 L of a hydroethanolic solvent (1: 1). The mixture was then hermetically covered and allowed to macerate at room temperature for 48 h. After this, the mixture was filtered and the resulting filtrate (Filtrate 1) was kept at 2°C. The remaining wet powder was once again extracted for 48 h under the same conditions as for the first extraction. This yielded a second filtrate (Filtrate 2). The two filtrates (1 and 2) were then mixed and smoothly evaporated at 50°C. This process led to a powder referred as hydroethanolic extract (HE) that was kept in a sealed flask at 2°C. The yield of the extraction was 21% (w/w).

In vitro measurement of pancreatic α-amylase activity

Pancreatic α-amylase (EC 3.2.1.1) activity was measured according to the kinetic method of Kruse-Jarres *et al.* (1989) using 4,6-ethylidene-(G7)-*p*-nitrophenyl-(G1)-α,D-maltoheptaoside (ethylidene-G$_7$PNP) as substrate. α-amylase-EPS reagents were obtained from Chronolab (Zug, Switzerland). Pancreatic α-amylase was obtained from Sigma-Aldrich (St. Louis, MO, USA) and prepared at a concentration of 15 mg/mL. Aliquots (30 μL) of water (control) or extract (test) were added to 20 μL of enzyme and 1 mL of the reconstituted substrate solution, gently mixed and incubated for 3 min at 25°C. The recorded rate of increase of absorbance at 405 nm due to the release of *p*-nitrophenol was used to determine the pancreatic α-amylase activity in the samples. The inhibition percentage was determined through the formula: Inhibition (%) = [ΔA_{405}/min (control) $-$ ΔA_{405}/min (test)] × 100/ ΔA_{405}/min (control). α-amylase activity was also measured through an endpoint iodine-starch assay (Komaki *et al.* 2003). In brief, 80 μL of water (control) or extract (test) were added to 20 μL of enzyme (30 μg/mL) and 1.3 mL of a Tris-HCl pH 6.8 buffer and gently mixed. The resulting mixture was preincubated at 30°C for 20 min. 100 μL of 15-min boiled potato starch (Sigma-Aldrich) at a concentration of 0.1% (w/v) were then added and the reaction was

allowed to occur at 30°C for 20 min. The reaction was finally stopped by adding 2 mL of an acidic iodine/potassium iodide solution. Optical densities were read at 580 nm against a blank. This assay was done in triplicate and a starch standard corresponding to the initial quantity of starch present in the medium before the enzyme reaction was also assessed. Inhibition percentages of α-amylase activity were determined through the following formula: Inhibition (%) = [A_{580} (test) $-$ A_{580} (control)] × 100/ [A_{580} (standard) $-$ A_{580} (control)].

Animal study

Cooked starch was prepared by dissolving 10 g of cornstarch (Sigma-Aldrich) in 100 mL of distilled water. The mixture was then boiled for 15 min with constant stirring and cooled at room temperature.

Thirty rats were fasted for 18 h prior to the experiment and were divided into six groups of five animals each in order to assess the oral starch tolerance (OST). The first group served as a reference and received glucose (0.2 g/100 g body weight (bw)) while the second group (control) received distilled water. The other four groups received the *G. brevis* extracts at doses of 250 and 500 mg/kg bw, respectively (test groups). 30 min later, cooked starch (0.1 g/100 g bw) was administered to all animals. All the products were given to rats through oral intubation. Blood samples were collected from the tail tip into tubes prewashed with heparin (400 U/mL) and sodium fluoride (0.1 mM) before centrifugation. Blood samples were withdrawn from each rat prior to starch loading and at 30, 60, 90 and 180 min following starch intubation.

After this experiment, control and test groups continued to receive distilled water and extracts respectively on a daily basis over a 4-week period in metabolic cages. The rats were provided with food and water *ad libitum*. Changes in body weight were monitored throughout the study on a weekly basis. Faeces was collected once a week over a 24-h period, weighed and dried to constant weight in an oven at 60°C.

Analytical techniques

Extract concentrations that inhibit the enzyme activity (IC$_{50}$) by 50% were determined through logarithmic regression ($0.92 < R^2 < 1$). Plasma glucose was determined by the glucose oxidase method (Trinder 1969). The plasma total incremental glucose (TIG) was calculated as the sum of changes in plasma glucose after correction for the baseline (time zero) following starch intubation (Madar 1989). The glycaemic index (GI) was calculated as the ratio of TIG in a given group to the TIG in the reference group multiplied by 100. The faecal water content was determined through the difference between fresh weight and dry weight. Results are expressed as means ± SD. Statistical analysis was carried out by Student's *t*-test (STT) and one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The software SPSS for Windows (SPSS Inc., Chicago, IL, USA) version 10.1 was used for the analysis. *P*-values less than 0.05 were considered as significant.

RESULTS

Effect of *Glyphaea brevis* on the *in vitro* activity of pancreatic α-amylase

Fig. 1 represents the *in vitro* inhibition profile of pancreatic α-amylase by the extracts of *G. brevis*. It appears that both aqueous (AE) and hydroalcoholic (HAE) extracts presented inhibitory effects toward the enzyme. These inhibitory effects were increasing along with the extract concentration in the different assays and the aqueous extract was the most effective in that way with an IC$_{50}$ of 6.71 ng/mL (vs. 7.85 μg/mL for HAE) for the kinetic method using ethylidene-G$_7$PNP as substrate (**Fig. 1A**) and an IC$_{50}$ of 0.74 mg/mL (vs. 2.43 mg/mL for HAE) for the endpoint method using starch as substrate (**Fig. 1B**). Enzyme inhibition was quite total with the first method while it did not exceed 60% in the iodine-starch assay.
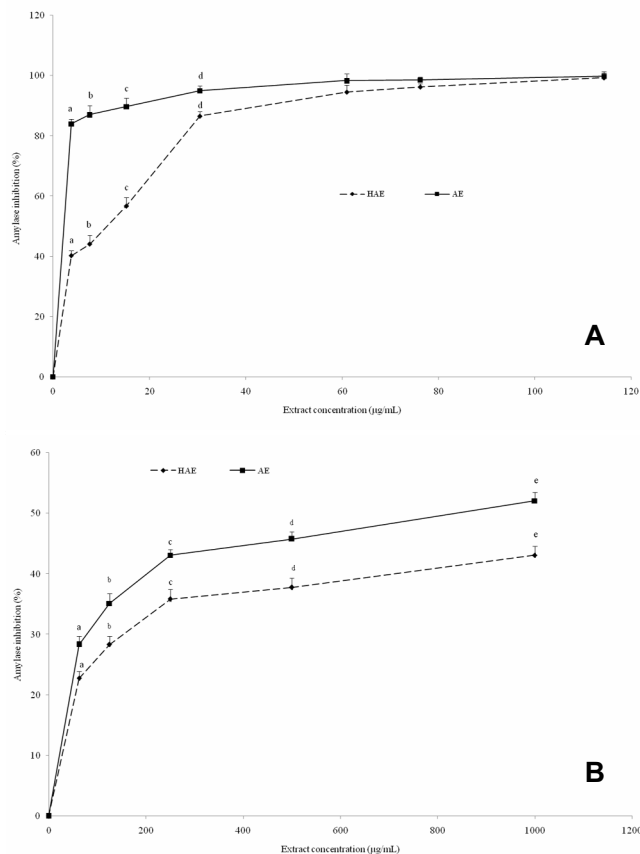
Fig. 1 Inhibition profile of pancreatic α-amylase by aqueous (AE) and hydroalcoholic (HAE) extracts of *Glyphaea brevis*. The substrates used are: (**A**) ethylidene-G$_7$PNP and (**B**) potato starch. Values sharing a common superscript letter differ significantly at $P<0.05$ (STT). (Means ± SD, $n = 5$).

Effect of *Glyphaea brevis* on oral starch tolerance (OST) in rats

Fig. 2 shows the changes (as a function of time) in postprandial plasma glucose after intubation of cooked starch to rats. There was no significant difference between the fasting plasma glucose levels in all the groups. A significant glycaemic peak was observed 30 min after the starch load in both reference (+237.88%, $p<0.005$) and control (+82.88%, $p<0.05$) groups. That peak was rather observed at time 60 min and reduced in groups treated with *Glyphaea brevis* extracts (AE 250 mg/kg: +51.85%, $p<0.05$; AE 500 mg/kg: +30.81%, $p<0.01$; HAE 250 mg/kg: +55.96%, $p<0.01$; HAE 500 mg/kg: +36.14%, $p<0.005$).

Table 1 compares the plasma total incremental glucose (TIG) and the glycaemic index (GI) of the cooked starch in rats from the different groups. Rats treated with the extracts at the dose of 500 mg/kg showed a significantly ($p<0.001$) lower TIG than that of reference and control groups, and, subsequently, a decrease in the GI of cooked starch. The aqueous extract was the most effective in inhibiting the

postprandial rise of plasma glucose with the maximum inhibition (42.17%) observed with the dose of 500 mg/kg. However, from a statistical point of view, the results were similar for both extracts.

Effect of *Glyphaea brevis* on body weight increase in rats

Fig. 3 presents the body weight increase of the rats from the different groups over a 4-week period. This body weight increase was significantly ($p<0.01$) lowered in the groups treated with the extracts by comparison with the control group. The difference in weight gain was visible in all the test groups from the first week until the end of the study. The lowest weight gain ($5.0 ± 1.2$ g) over four week was observed in the group receiving the hydroalcoholic extract at the dose of 500 mg/kg. For each extract, the weight control effect appeared to be dose-dependent.
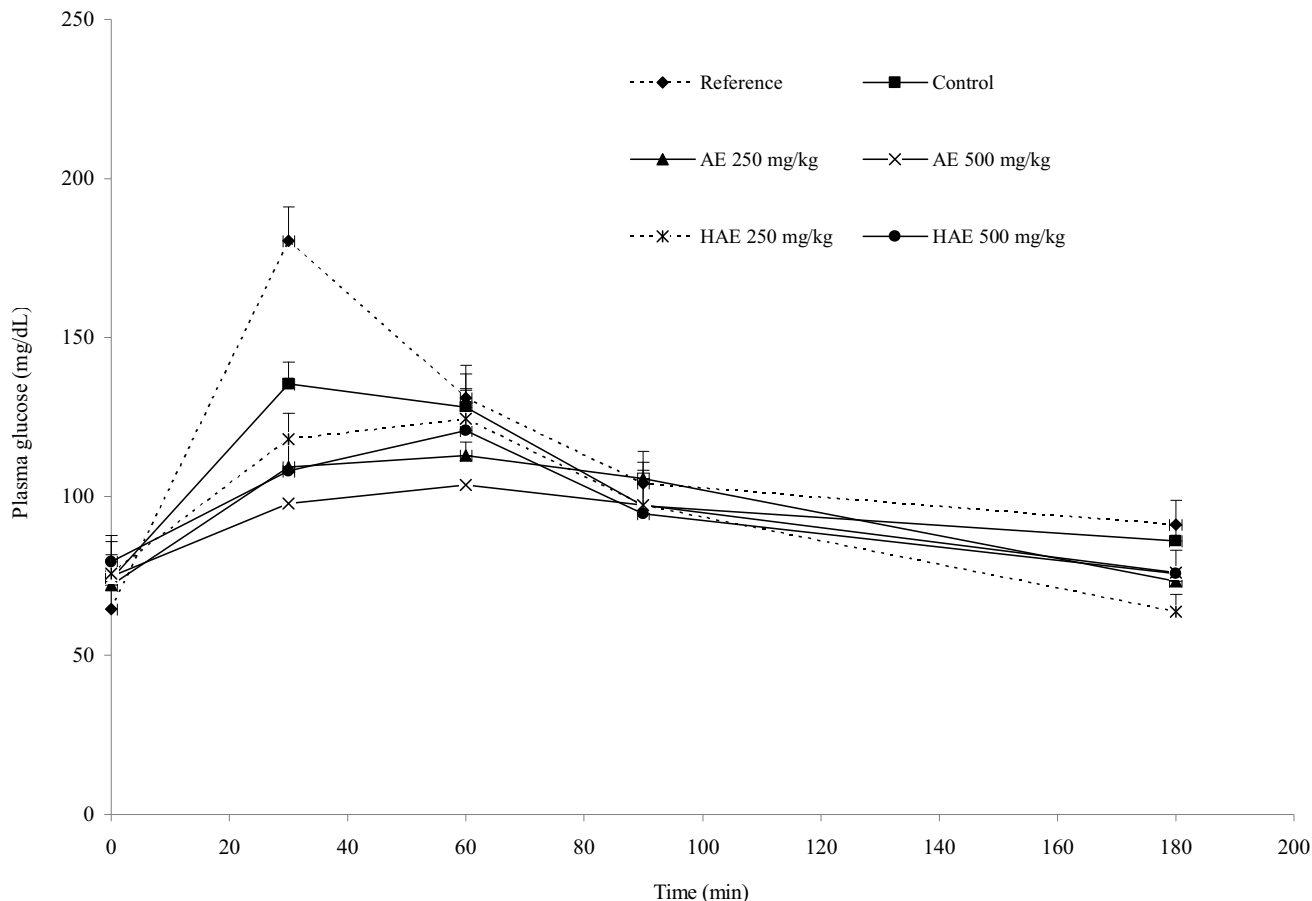
Effect of *Glyphaea brevis* on faecal weight in rats

Table 2 is comparing the faecal weight (fresh and dry) of the animals from all the groups. Both fresh and dry faecal weight were significantly ($p<0.05$) higher in the test groups than in the control group while there were minor changes in the faecal water content.

DISCUSSION

Both aqueous and hydroalcoholic extract of *Glyphaea brevis* had inhibitory effects on the activity of α-amylase (**Fig. 1**). Comparison of IC$_{50}$ values showed that the aqueous extract was the most effective in that enzyme inhibition. This differential reactivity could be the result of the different proportions of the compounds present in each extract. The inhibition percentage of α-amylase by *Glyphaea brevis* extracts was higher with ethylidene-G$_7$PNP than with starch. This difference could be due to structural differences existing between both substrates as suggested by Al-Dabbas *et al.* (2006). However, it is interesting to note that the kinetic assay using ethylidene-G$_7$PNP as substrate also involves α-(1,6)-glucosidase to release *p*-nitrophenol that is measured spectrophotometrically (Kruse-Jarres *et al.* 1989). Therefore, the possibility of an inhibition of this enzyme should not be excluded.

One of the metabolic effects of α-amylase inhibitors is the reduction of the peak postprandial blood glucose, as observed with the well-known wheat and *Phaseolus vulgaris* amylase inhibitors (Lankisch *et al.* 1998; Obiro *et al.* 2008). Therefore, the delay and the decrease in postprandial plasma glucose observed in the extract treated groups during the OST study would not merely be the fact of a delayed and/or reduced glucose absorption since the enzyme-inhibiting activity of the extracts has been established *in vitro*. This inhibition led to a decrease of the glycaemic index of cornstarch as confirmed by the TIG values in all groups (**Table 1**).

Limiting glucose availability is of interest for obese patients. In fact, glucose has been identified a substrate for endogenous lipogenesis (Foufelle and Ferré 2004) and the rate of lipogenesis has been reported to be increased in the

Table 1 Comparison of the plasma total incremental glucose (TIG) and the glycaemic index (GI) of cornstarch in rats treated with aqueous (AE) and hydroalcoholic (HAE) extracts of *Glyphaea brevis*. (Means ± SD, $n = 5$).

Group	Dose (mg/kg)	TIG (mg/dL)	GI (%)	Inhibition (%)
Without treatment				
Reference	-	256.25 ± 19.60 a	100	-
Control	-	134.33 ± 21.87 b	52.42 ± 5.01 a	-
Treated				
AE	250	116.00 ± 18.93 b	45.27 ± 7.29 a	13.65 ± 0.05 a
	500	77.67 ± 14.08 c	30.31 ± 5.86 b	42.17 ± 1.28 b
HAE	250	112.67 ± 19.44 b	43.71 ± 7.69 a	16.12 ± 0.97 c
	500	86.33 ± 12.88 c	33.69 ± 4.65 b	35.73 ± 1.04 d

In a column, values not sharing the same superscript letter differ significantly at $p<0.05$ (DMRT).

	30	60	90	180 min
AE 250 mg/kg	*p*=0.01	-	-	*p*=0.01
AE 500 mg/kg	*p*<0.005	-	*p*<0.05	-
HAE 250 mg/kg	*p*<0.05	-	-	*p*<0.01
HAE 500 mg/kg	*p*=0.01	-	-	*p*<0.05

Fig. 2 Comparison of changes in postprandial plasma glucose after intubation of cornstarch to rats treated with aqueous (AE) and hydroalcoholic (HAE) extracts of *Glyphaea brevis*. Reference animals were intubated with glucose at 2 g/kg body weight. (Means ± SD, *n* = 5). Compared to the control, values were significantly different as mentioned above.

Table 2 Comparison of faecal weight (fresh and dry) and faecal water content (FWC) of rats treated with aqueous (AE) and hydroalcoholic (HAE) extracts of *Glyphaea brevis* over 4 weeks. (Means ± SD, *n*=5)

Time point	Group	FFW (g/24 h/rat)	DFW (g/24 h/rat)	FWC (%)
Baseline	Control	2.4 ± 0.2 a	1.4 ± 0.6 a	45.36 ± 16.74 a
	AE 250 mg/kg	2.8 ± 0.5 a	1.6 ± 0.2 a	42.48 ± 3.18 a
	AE 500 mg/kg	2.3 ± 0.2 a	1.3 ± 0.1 a	43.05 ± 8.42 a
	HAE 250 mg/kg	2.5 ± 0.4 a	1.4 ± 0.2 a	43.39 ± 9.42 a
	HAE 500 mg/kg	2.8 ± 0.6 a	1.6 ± 0.3 a	42.63 ± 1.61 a
Week 1	Control	3.1 ± 0.3 a	1.6 ± 0.1 a	48.27 ± 1.78 a
	AE 250 mg/kg	4.6 ± 0.4 b	2.7 ± 0.1 b	41.13 ± 2.96 b
	AE 500 mg/kg	6.2 ± 0.5 c	3.8 ± 0.1 c	38.53 ± 3.36 b
	HAE 250 mg/kg	4.2 ± 0.2 b	2.7 ± 0.0 b	36.62 ± 3.07 b
	HAE 500 mg/kg	4.7 ± 0.6 b	2.9 ± 0.2 b	37.98 ± 3.69 b
Week 2	Control	3.2 ± 0.3 a	2.2 ± 0.1 a	31.04 ± 3.35 a
	AE 250 mg/kg	4.3 ± 0.5 b	2.4 ± 0.3 ac	43.13 ± 13.68 ab
	AE 500 mg/kg	7.5 ± 0.6 c	3.8 ± 0.2 b	48.97 ± 6.77 b
	HAE 250 mg/kg	3.4 ± 0.4 a	2.0 ± 0.1 a	40.86 ± 4.04 b
	HAE 500 mg/kg	4.4 ± 0.2 b	2.6 ± 0.1 c	40.75 ± 4.97 b
Week 3	Control	5.7 ± 0.2 a	3.0 ± 0.2 a	47.24 ± 5.36 a
	AE 250 mg/kg	4.7 ± 0.1 b	2.6 ± 0.1 b	44.63 ± 3.30 ab
	AE 500 mg/kg	6.3 ± 0.4 c	3.5 ± 0.2 c	44.16 ± 6.73 ab
	HAE 250 mg/kg	4.8 ± 0.2 d	3.1 ± 0.1 a	35.28 ± 4.78 b
	HAE 500 mg/kg	5.1 ± 0.7 abd	3.1 ± 0.3 ac	38.67 ± 8.12 ab
Week 4	Control	3.8 ± 0.4 a	2.2 ± 0.3 a	41.92 ± 7.46 a
	AE 250 mg/kg	5.1 ± 0.4 b	3.1 ± 0.2 b	39.06 ± 4.62 a
	AE 500 mg/kg	6.9 ± 0.6 c	3.8 ± 0.5 b	45.07 ± 2.48 a
	HAE 250 mg/kg	5.3 ± 0.4 b	3.2 ± 0.4 b	39.77 ± 3.01 a
	HAE 500 mg/kg	5.8 ± 0.5 b	3.4 ± 0.2 b	41.18 ± 4.72 a

In a column and for each time point, values not sharing the same superscript letter differ significantly at *p*<0.05 (DMRT).
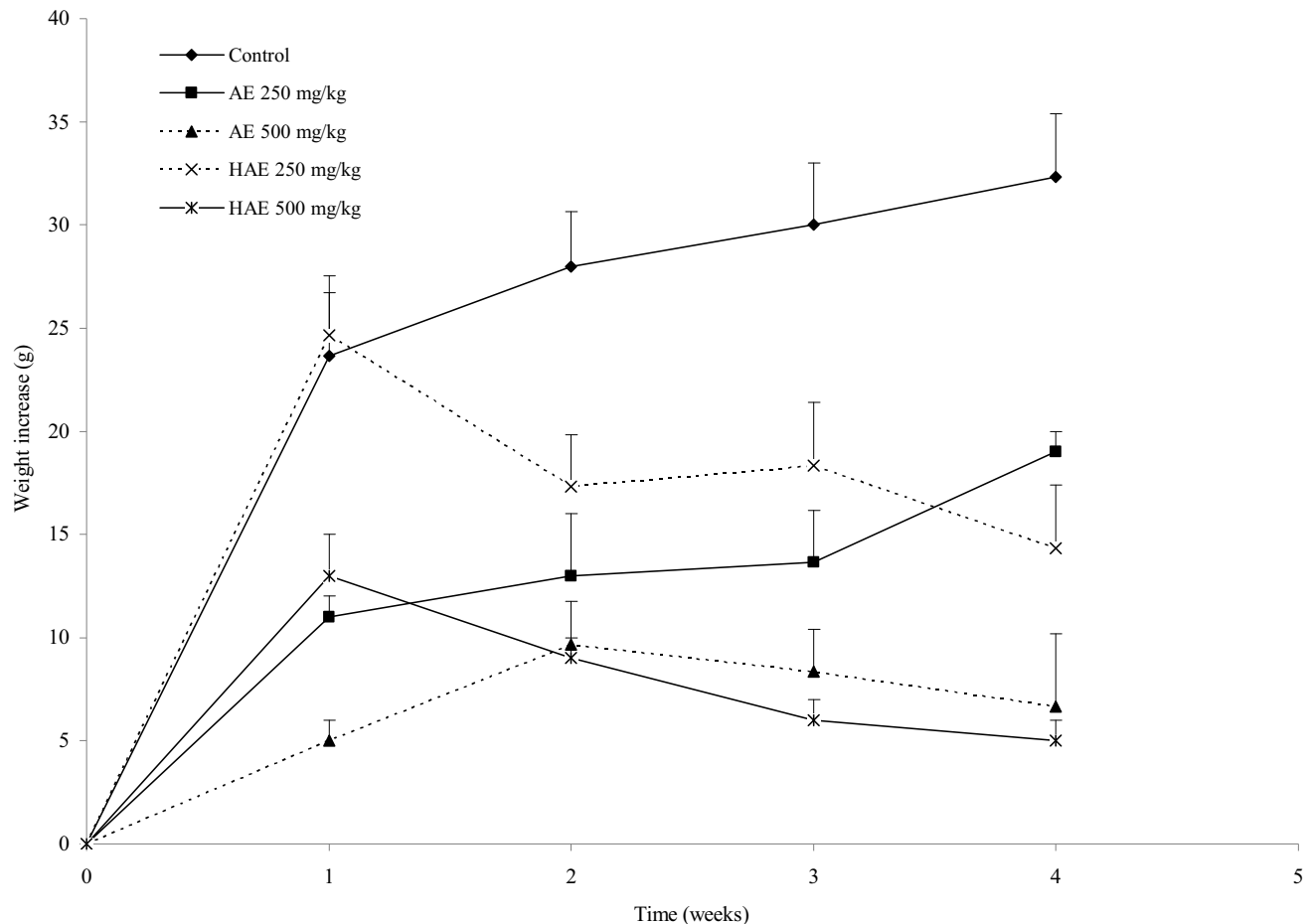
	1	2	3	4 weeks
AE 250 mg/kg	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
AE 500 mg/kg	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
HAE 250 mg/kg	-	$p<0.001$	$p<0.01$	$p<0.001$
HAE 500 mg/kg	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$

Fig. 3 Comparison of the body weight increase of rats treated with aqueous (AE) and hydroalcoholic (HAE) extracts of *Glyphaea brevis* over 4 weeks. (Means ± SD, *n* = 5). Compared to the control, values were significantly different as mentioned above.

adipose tissue of obese patients (Diraison *et al.* 2002). Therefore, inhibition of starch digestion may be assimilated to an upstream inhibition of lipogenesis and as a strategy to prevent and/or control obesity. The lower weight gain noted in groups treated with the extracts is an illustration of the weight loss/weight control properties of "starch blockers" (Thom 2000). Standard chow diets for rodents are mainly made of carbohydrates represented by starches. Therefore, the weight control in our study would be assured through the reduction of glucose availability from dietary starch. This seems to be confirmed by the results drawn from faecal weight monitoring where the dry faecal weight was significantly increased in groups receiving the extracts by comparison with the control group (**Table 2**). Moreover, no significant difference was noted in the food intake in all groups (data not shown). Thus, inhibition of starch digestion would trigger a greater faecal excretion of nutrients that would result in a higher faecal weight as noted by some authors (Fölch *et al.* 1981).

Another potential use for these amylase inhibitors is the control of blood sugar levels. In fact, α-amylase inhibitors reduce the glucose peaks that can occur after a meal, slowing the speed with which α-amylase can convert starch to simple sugars until the body can deal with it. This is of particular importance in people with diabetes, where low insulin levels prevent extracellular glucose from being cleared quickly from the blood. Several amylase-inhibiting drugs (e.g., acarbose, miglitol) are in use to reduce postprandial glucose in diabetic patients, often in conjunction with insulin (Madar 1989).

Thus, our study shows that *Glyphaea brevis* Spreng. (Monach.) could be effective in the control of both body weight and postprandial blood glucose due to its pancreatic α-amylase inhibitory properties coupled to its ability to increase stool weight. This finding may find applications in the areas of human obesity management and weight loss.

REFERENCES

Adjanohoun JE, Aboubakar N, Dramane K, Ebot ME, Ekpere JA, Enowora EG, Focho D, Ogbite Z, Kamanyi A, Kamsu-Kom J, Keita A, Mbenkum T, Mbi CN, Mbiele AL, Mbome IL, Muburu NK, Nancy WL, Nkongmeneck B, Satabie B, Sofowora A, Tamze V, Virmum CK (1996) *Traditional Medicine and Pharmacopeia. Contribution of Ethnobotanical and Fleuristic Studies in Cameroon*, Centre National de Production de Manuels Scolaires, Porto Novo, Benin, 393 pp

Alam N, Gourinath S, Dey S, Srinivasan A, Singh TP (2001) Substrate-inhibitor interactions in the kinetics of α-amylase inhibition by Ragi α-amylase/trypsin inhibitor (RATI) and its various N-terminal fragments. *Biochemistry* **40**, 4229-4233

Al-Dabbas MM, Kitahara K, Suganuma T, Hashimoto F, Tadera K (2006) Antioxidant and α-amylase inhibitory compounds from aerial parts of *Varthemia iphionoides* Boiss. *Bioscience, Biotechnology and Biochemistry* **70**, 2178-2184

Bhat RB, Eterjerer EO, Oladipo VT (1990) Ethnobotany studies from central Nigeria. In: **Ogbonnia S, Van Staden J, Jager AK, Coker HA** (2003) Anticonvulsivant effect of *Glyphaea brevis* (Speng.) Moraches leaf extracts in mice and preliminary phytochemical tests. *Nigerian Quarterly Journal of Health and Medicine* **13 (3-4)**, 62-64

Bovet P, Chiolero A, Paccaud F (2008) Epidemiology and prevention of obesity in children and adolescents (in French). *Revue Médicale Suisse* **4 (148)**, 650-652, 654-656

Bray GA, Greenway FL (1999) Current and potential drugs for treatment of obesity. *Endocrinology Review* **20**, 805-79

Choudhury A, Maeda K, Murayama R, DiMagno EP (1996) Character of a wheat amylase inhibitor preparation and effects on fasting human pancreatic-cobiliary secretions and hormones. *Gastroenterology* **111**, 1313-20

Dakam W, Shang J, Agbor G, Oben J (2007) Effects of sodium bicarbonate and albumin on the *in vitro* water-holding capacity and some physiological properties of *Trigonella foenum graecum* L. galactomannan in rats. *Journal of Medicinal Food* **10**, 169-174

Diraison F, Dusserre E, Vidal H, Sothier M, Beylot M (2002) Increased hepatic lipogenesis but decreased expression of lipogenic gene in adipose tissue in human obesity. *American Journal of Physiology, Endocrinology and Metabolism* **282**, E46-E51

Fatumbi VP (1995) Ewé, the use of plants in Yoruba Society. In: Adams M, Gmünder F, Hamburger M (2007) Plants traditionally used in age related brain disorders – A survey of ethnobotanical literature. *Journal of Ethnopharmacology* **113**, 363-381

Finkelstein EA, Trogdon JG, Brown DS, Allaire BT, Dellea PS, Kamal-Bahl SJ (2008) The lifetime medical cost burden of overweight and obesity: Implications for obesity prevention. *Obesity* **16**, 1843-1848

Fölsch UR, Grieb N, Caspary WF, Creutzfeldt W (1981) Influence of short- and long-term feeding of an alpha-amylase inhibitor (BAY e 4609) on the exocrine pancreas of the rat. *Digestion* **21**, 74-82

Foufelle F, Ferré P (2004) Nutrition, fat synthesis and obesity. In: Remacle C, Reusens B (Eds) *Functional Foods, Ageing and Degenerative Disease*, CRC Press, Boca Raton, FL, pp 260-277

Garrow JS, Scott PF, Heels S, Nair KS, Halliday D (1983) A study of "starch blockers" in man using ^{13}C-enriched starch as a tracer. *Human Nutrition and Clinical Nutrition* **37**, 301-305

Hudgins LC, Hellerstein MK, Seidman CE, Neese RA, Tremaroli JD, Hirsch J (2000) Relationship between carbohydrate-induced hypertriglyceridemia and fatty acid synthesis in lean and obese subjects. *Journal of Lipid Research* **41**, 595

Jenkins DJA, Wolever TMS, Leeds AR, Gassull MA, Harsman P, Diliwari J, Goff DV, Metz GL, Alberti KGM (1978) Dietary fibres, fibre analogues, and glucose tolerance: importance of viscosity. *British Medical Journal* **I**, 1392-1394

Komaki E, Yamaguchi S, Maru I, Kinoshita M, Kakehi K, Ohta Y, Tsukuda Y (2003) Identification of anti- α-amylase components from olive leaf extracts. *Food Science and Technology Research* **9**, 35-39

Kruse-Jarres JD, Kaiser C, Hafkenscheid JC, Hohenwallner W, Stein W, Bohner J, Klein G, Poppe W, Rauscher E (1989) Evaluation of a new alpha-amylase assay using 4.6-ethylidene-(G7)-1-4-nitrophenyl-(G1)-alpha-D-maltoheptaoside as substrate. *Journal of Clinical Chemistry and Clinical Biochemistry* **27**, 103-113

Lankisch M, Layer P, Rizza RA, DiMagno EP (1998) Acute postprandial gastrointestinal and metabolic effects of wheat amylase inhibitor (WAI) in normal, obese, and diabetic humans. *Pancreas* **17**, 176-181

Madar Z (1989) The effect of acarbose and miglitol (BAY-M-1099) on postprandial glucose levels following ingestion of various sources of starch by nondiabetic and streptozotocin-induced diabetic rats. *Journal of Nutrition* **199**, 2023-2029

Marshall JJ, Lauda CM (1975) Purification and properties of phaseolamin, an inhibitor of alpha-amylase, from the kidney bean, *Phaseolus vulgaris*. *The Journal of Biological Chemistry* **250**, 8030-8037

McDougall G, Shipro F, Dobson P, Smith P, Blake A, Stewart D (2005) Different polyphenolic components of soft fruits inhibit α-amylase and α-glucosidase. *Journal of Agricultural and Food Chemistry* **53**, 2760-2766

Obiro WC, Zhang T, Jiang B (2008) The nutraceutical role of the *Phaseolus vulgaris* alpha-amylase inhibitor. *British Journal of Nutrition* **100**, 1-12

Ogbonnia S, van Staden J, Jager AK, Coker HA (2003) Anticonvulsivant effect of *Glyphaea brevis* (Speng.) Moraches leaf extracts in mice and preliminary phytochemical tests. *Nigerian Quarterly Journal of Health and Medicine* **13 (3-4)**, 62-64

Ogden CL, Carrol MD, Curtin LR, McDavell MA, Tabak CJ, Flegal KM (2006) Prevalence of overweight and obesity in the United States, 1999-2004. *Journal of the American Medical Association* **295**, 1549-1555

Queseda C, Bartolome B, Nieto O, Gomez-Cordoves C, Hernandez T, Estrella I (1995) Phenolic inhibitors of α-amylase and trypsin enzymes by extracts from pears, lentils and coca. *Journal of Food Protection* **59**, 185-192

Terashima H, Ichikawa M (2003) A comparative ethnobotany of the Mbuti and Efe hunter-gatherers in the Ituri forest, Democratic Republic of Congo. *African Study Monographs* **24 (1, 2)**, 1-168

Thom E (2000) A randomized, double-blind, placebo-controlled trial of a new weight-reducing agent of natural origin. *Journal of International Medical Research* **28**, 229-233

Trinder P (1969) Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Annals of Clinical Biochemistry* **6**, 24

Umoren J, Kies C (1992) Commercial soybean starch blocker consumption: impact on weight gain and on copper, lead and zinc status of rats. *Plant Foods and Human Nutrition* **42**, 135-142

World Health Organization (1997) *Obesity; Preventing and Managing the Global Epidemic*; Report of a WHO consultation on obesity, World Health Organization: Geneva, Switzerland, 16 pp

World Health Organization (2006) *Obesity and Overweight*, Factsheet no. 311, World Health Organization: Geneva, Switzerland, 1 p