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Intestinal disaccharidases and some renal enzymes in streptozotocin-induced diabetic rats fed sapogenin extract from bitter yam (*Dioscorea polygonoides*)

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Abstract

In this study, the effects of bitter yam sapogenin extract or commercial diosgenin on intestinal disaccharidases and some renal enzymes in diabetic rats were investigated. Diabetic male Wistar rats were fed diets supplemented with 1% sapogenin extract or commercial diosgenin for 3 weeks. Plasma glucose, intestinal disaccharidases and the activities of transaminases, acid phosphatase, glucose-6-phosphatase, ATP citrate lyase, glucose-6-phosphate dehydrogenase and pyruvate kinase were assessed for the level of metabolic changes in the kidney of diabetic rats. Sapogenin extract or commercial diosgenin supplementation resulted in a significant decrease in lactase and maltase activities in all three regions of the intestine compared to the diabetic control group. However, the test diets significantly reduced intestinal sucrase activity in the proximal and mid regions. Test diets supplementation resulted in a significant decrease in the activities of the transaminases compared to the normal and diabetic control groups. The activity of glucose-6-phosphatase was significantly increased while the activities of ATP citrate lyase, pyruvate kinase and glucose-6-phosphate dehydrogenase were significantly reduced in the kidney of the diabetic control rats compared to the normal group. Test diets supplementation did not significantly alter glucose-6-phosphatase, ATP citrate lyase and pyruvate kinase activities compared to the diabetic control. However, there was a significant increase in glucose-6-phosphate dehydrogenase activity toward the normal group. In conclusion, the consumption of bitter yam sapogenin extract or commercial diosgenin demonstrated hypoglycemic properties, which are beneficial in diabetes by reducing intestinal disaccharidases activities; however, bitter yam sapogenin extract may adversely affect the integrity of kidney membrane.

Keywords: Diabetes; Disaccharidases; Streptozotocin; Intestine; Kidney; Sapogenin; Diosgenin

Introduction

Yam is the leading form of staple for millions of people in the tropical and subtropical countries. All the yams that are of economic importance as food crops are tuberous, and it is this tuber that contains the plant food reserves, mainly starch, that is often incorporated in the human diet (Coursey, 1967). Research has shown that some species of yam contain significant levels of secondary metabolites that may produce toxic effects in rats (Prima and Kuriup, 1979; Panigrahi and Francis, 1982; Tan et al., 2003). The toxic compounds present in some yam varieties are utilized in the manufacturing of insecticidal powder and poisons for fowls and fishes (Purseglove, 1972; Coursey, 1967). The tubers are also rich sources of steroidal saponins and often serve as raw material in the manufacturing of sex hormones, cortisone and contraceptives (Purseglove, 1972).

The wild yam species in Jamaica are *Dioscorea polygonoides* (wild yam, bitter yam or bitter Jessie), *Dioscorea dumentorum* (bitter or cluster yam), *Rajania ovate* and *Rajania cordata* (himber). The bitter Jessie as the name suggests, has a characteristic bitter taste and may be confused with *D. dumentorum.* The main morphological difference between the two yams lies in the leaf structure. The bitter/cluster yam has a trifoliate leaf while the bitter Jessie has simple, light green, glossy leaves. Bitter yam (*D. polygonoides*) is sometimes used to make "roots tonic" in Jamaica. We have earlier reported high level of sapogenin in bitter yam compared to other yam varieties in Jamaica (Asemota et al., 2003). There are several reports showing the hypoglycemic effect of diosgenin in normal and diseased states (Prasanna, 2000; Sharma et al., 1990; Cayen and Dvornik, 1979). The consumption of sapogenin extract from

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bitter yam has been demonstrated to be beneficial by lowering blood glucose and lipid profile in streptozotocin-induced diabetic rats (McAnuff et al., 2002). However, the mechanism by which sapogenin extract reduces blood glucose is not known. The effects of consumption of sapogenin extract on kidney metabolism have not been investigated.

We have investigated the effects of sapogenin extract from bitter yam on blood glucose together with the activities of small intestinal disaccharidases and we have also examined the effects of the supplement on kidney metabolism in streptozotocin-induced diabetic rats. Commercial diosgenin supplement at the same level was used for comparison purposes.

Methods

Diosgenin was purchased from Sigma-Aldrich (St. Louis, MO) and the normal diet (PMI Feeds Inc. Lab Diet #5001) was a marketed laboratory rodent diet recommended for rats, mice and hamsters with the approximate chemical composition: protein 23%, fat 4.5%, fibre 6.0%, ash 8.0% and carbohydrate 58.5%. All other reagents were of analytical grade.

Sample preparation

Freshly harvested tubers of bitter yam (*D. polygonoides*) were obtained from Muirhouse, St. Ann, Jamaica. Sapogenin was extracted from Jamaican bitter yam using the method of Morris et al. (1958). Freshly harvested tubers (50 g) were peeled, chopped and refluxed with 3.5 M HCl (115 ml) for 3 h. The solution was filtered and the residue was washed with water to neutrality. The filter paper and residue were dried at 65–70 °C overnight and then extracted with petroleum ether in a Soxhlet apparatus for 6 h. The petroleum ether extract was concentrated in vacuo. The solid that precipitated was filtered and dried to give the crude sapogenin extract.

The sapogenin extract or commercial diosgenin (purchased from Sigma-Aldrich, St. Louis, MO) were supplemented at a level of 1.0 g of sapogenin extract (80% diosgenin and the other 20% made up of β-sitosterol, pennogenin, stigmasterol and $\Delta\Delta^3$ diosgenin) or commercial diosgenin/100 g of rat basal diet (Cayen and Dvornik, 1979).

Animals

Adult male Wistar rats (32) obtained from the University of the West Indies Animal House were divided into four groups by weight for a 3-week study (eight rats per group, average body weight 249.0±0.3 g). The experimental groups were as follows: non-diabetic rats receiving normal diet (normal), diabetic rats fed normal diet (diabetic), diabetic rats fed sapogenin extract (sapogenin extract) and diabetic rats fed commercial diosgenin (diosgenin).

Diabetes was induced by a single intraperitoneal injection of streptozotocin (60 mg/kg body weight in 0.05 M citrate buffer, pH 4.5). The normal control group was injected intraperitoneally with an equivalent amount of buffer (0.05 M citrate buffer, pH 4.5). After 8 days, blood was withdrawn from the tail vein and the level of blood glucose determined using the method of Teller (1956). Diabetes was confirmed when blood glucose was about four times in excess of normal. Initial blood glucose for normal rats was 4.77±0.75 mmol/L, while the initial blood glucose for all three diabetic groups ranged from 17.04±0.98 to 32.52±4.49 mmol/L.

Rats were housed in stainless steel cages in a room kept on a 12-h light–dark cycle, and were allowed to have access to their respective diets and water ad libitum. The cages were cleaned daily. Body weight change and total food intake were recorded weekly. The rats were fed on their respective diets for 21 days and sacrificed by decapitation after an overnight fast. Approval for the study was obtained from the Board of Ethical Studies, Faculty of Medical Sciences, University of the West Indies, Mona. Blood was withdrawn from the tail vein and used for plasma glucose determination. Intestine and kidney samples were excised, weighed, frozen in liquid nitrogen and assayed for enzyme activities.

Metabolite assays

Blood glucose was determined using the method of Teller (1956).

Enzyme assays

The intestine of each rat was divided into three portions: the proximal (duodenum), mid (jejunum) and distal (ileum). Rat intestine, which was free of food materials, was excised and the lumen was flushed out several times with 0.9% NaCl. The mucosal washing and the scraped mucosa were pooled, homogenized, centrifuged (5000×*g*) and the supernatant was frozen until required for enzymatic assays. The activities of intestinal disaccharidases were obtained by measuring the amount of glucose released from various substrates (Dahlqvist,

Table 1
Body weight changes, feed intake, kidney weight and final plasma glucose of rats fed bitter yam sapogenin extract or commercial diosgenin supplements

	Normal control	Diabetic control	Sapogenin extract	Commercial diosgenin
Final body weight (g)	325.4±9.2[a]	247.3±27.0[b]	190.8±17.4[b]	193.3±8.8[b]
Initial body weight (g)	248.4±16.2[a]	249.3± 9.2[a]	249.8±14.1[a]	248.4±16.8[a]
Daily feed intake (g)	14.6±0.0[a]	14.5±0.1[a]	14.6±0.1[a]	14.4±0.1[a]
Kidney weight (g)	2.8±0.1[a]	2.7±0.2[a]	2.2±0.2[a]	2.6±0.2[a]
Intestine weight (g)	8.6±0.5[a]	6.5±0.4[b]	5.0±0.2[c]	6.3±0.2[b]
Final plasma glucose (mM/L)	5.9±0.4[a]	29.6±2.2[b]	18.2±0.7[c]	19.7±0.7[c]

Values expressed as mean±S.E.M. Figures in rows with different letter superscripts are significantly different ($P<0.05$).