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Hypoglycemic effects of steroidal sapogenins isolated from Jamaican bitter yam, *Dioscorea polygonoides*

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Abstract

In this study, three steroidal sapogenins (Δ^3 diosgenin, diosgenin, and pennogenin) and the phytosterols, stigmasterol and β-sitosterol were isolated from Jamaican bitter yam, *Dioscorea polygonoides*. Their effects on fasting blood glucose and intestinal amylase and ATPases in streptozotocin-induced diabetic rats were studied.

The diabetic rats (fed supplemented and unsupplemented diets) lost weight significantly compared to the normal group. There was a significant increase in the activity of α-amylase in the proximal region of the small intestinal mucosa of diabetic rats fed sapogenin extract or commercial diosgenin. However, this did not result in increased fasting blood glucose. Instead, supplementation of the diet with bitter yam sapogenin extract significantly decreased fasting blood glucose compared to the diabetic group. Supplementation of the diet with bitter yam sapogenin extract or commercial diosgenin significantly reduced Na^+–K^+-ATPase activity in all three regions compared to the diabetic control group. Commercial diosgenin supplementation resulted in a significant increase in Ca^{2+} ATPase activity in proximal region compared to the diabetic control and bitter yam sapogenin extract groups. The effect of bitter yam sapogenin extract or commercial diosgenin on intestinal Na^+–K^+-ATPase activity could account for their hypoglycemic properties. However, there was adverse effect on the body weight.

Keywords: Sapogenin; Hypoglycemia; Intestinal ATPases; *Dioscorea polygonoides*

1. Introduction

Diabetes mellitus is a clinical syndrome characterized by elevated plasma glucose levels resulting from absolute or relative insulin deficiency. The prevalence of the different types of diabetes varies in different parts of the world. In Jamaica, the point prevalence of diabetes mellitus in the 15 and over age group is estimated to be 17.9% (Ragoobirsingh et al., 1995). It is projected that by 2010, at least 239 million people will be affected by this disease globally (Mandrup-Poulsen, 1998). The high prevalence of diabetes mellitus as well as its long-term complications has led to an ongoing search for hypoglycemic agents from natural sources (Alarcon-Aguilar et al., 1997).

Yams are angiosperms, or flowering plants (Coursey, 1967), and are monocotyledons belonging to the family Dioscoreaceae within the Order Dioscoreales. Although the genus *Dioscorea* consists of over 600 species worldwide, only 10 are edible. Apart from the edible yams, the family Dioscoreaceae contains many species that are of interest due to their medicinal or toxic properties (Coursey, 1967; Akahori et al., 1969). Several species are known to be pharmacologically active against diabetes

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mellitus, hypertension and other physical ailments (Undie and Akubue, 1986; Kaimal and Kemper, 1999). Akubue and Mittal (1982) reported the use of the *Dioscorea dumentorum* tuber by a Nigerian herbalist in the treatment of clinical diabetes.

To date, the anti-diabetic property of the Jamaican bitter yam has not been studied. Hence, this study was undertaken to chemically characterize the sapogenins present and to assess the effects of their consumption on blood glucose.

2. Materials and methods

2.1. Plant material

Freshly harvested tubers of bitter yam (*Dioscorea polygonoides*) were obtained from St. Ann, Jamaica. A sample was deposited at the University of the West Indies Herbarium (Voucher specimen #34576).

2.2. Preparation of sapogenin extract

The isolation of the crude sapogenin extract was performed as described by 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, the residue washed with water to neutrality and the filter paper and residue dried at 65–70 °C overnight. The dried residue was then extracted with petroleum ether in a Soxhlet apparatus for 6 h and the petroleum ether extract concentrated in vacuo. The resulting solid which precipitated was filtered and dried to give the crude sapogenin extract (yield: 0.2% on a fresh weight basis).

2.3. Isolation of sapogenins

Crude sapogenin extract of *D. polygonoides* was chromatographed on a silica gel column (5 cm × 20 cm) eluting with *n*-hexane:ethyl acetate (80:20), and 50 ml fractions were collected. The purity of the fractions collected was analyzed by thin layer chromatography (TLC) on silica gel developed in *n*-hexane:ethyl acetate (70:30). Spots were visualized by spraying the plates with phosphomolybdic acid spray reagent. Individual fractions were combined on the basis of their TLC profiles. Repeated column chromatography of the pooled fractions led to the isolation of three pure compounds and an inseparable mixture. Melting points were recorded on a Thomas–Hoover capillary melting point apparatus. FTIR spectra were recorded on a Perkin–Elmer 735B spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ACE 200 spectrometer with TMS as internal standard.

2.4. Experimental animals

Adult wistar rats (32) obtained from the University of the West Indies Animal House were divided into four groups by weight for a three-week study (8 rats per group, average body weight 249.0 ± 0.3 g). The groups were composed as follows: Nondiabetic rats receiving normal diet (normal); diabetic rats fed normal diet (diabetic); diabetic rats fed 1% of bitter yam steroidal sapogenin extract (sapogenin extract) and diabetic rats fed 1% commercial diosgenin-purchased from Sigma–Aldrich, St. Louis, MO (commercial diosgenin).

Three of the four groups received a single injection of streptozotocin (Sigma, 60 mg/kg-body weight in 0.05 M-citrate buffer, pH 4.5) intraperitoneally. The fourth group, 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 obtained from the tail vein and the level of fasting blood glucose determined using the method of Trinder (1969). Initial fasting blood glucose levels for normal rats was 4.77 ± 0.75 mM/l, while the initial fasting blood glucose for diabetic rats and those that received sapogenin extract or diosgenin supplements ranged from 17.04 ± 0.98 to 32.52 ± 4.49 mmol/l.

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%.

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. Changes in body weight 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 after the presentation of the protocol to the Board of Ethical Studies, Faculty of Medical Sciences, University West Indies, Mona.

2.5. Biochemical assays

The intestine of each rat 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.

2.5.1. Determination of intestinal amylase activity

Amylase activity was determined by measuring the amount of starch hydrolysed (Wotton, 1964).