Changes in Some Liver Enzymes in Streptozotocin-induced Diabetic Rats fed Sapogenin Extract from Bitter Yam (*Dioscorea polygonoides*) or Commercial Diosgenin

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ABSTRACT

The effects of steroidal sapogenin extract from bitter yam or commercial diosgenin on liver enzyme changes were investigated. Diabetic male Wistar rats were fed diets supplemented with 1% steroidal sapogenin extract or commercial diosgenin for three weeks. Plasma glucose levels and the activities of hepatic glucose-6-phosphatase, pyruvate kinase and glucose-6-phosphate dehydrogenase were assessed. Liver total cholesterol, HDL-cholesterol and total phospholipid were also measured. Plasma glucose decreased significantly ($p < 0.05$) in diabetic rats fed the three test diets compared to the diabetic control. The three test diets significantly decreased glucose-6-phosphatase activity compared to the diabetic control. The activities of ATP-citrate lyase, pyruvate kinase and glucose-6-phosphate dehydrogenase were significantly reduced in the liver of diabetic rats compared to normal control. Supplementation of the diet with bitter yam steroidal sapogenin extract or commercial diosgenin did not significantly alter ATP citrate lyase and pyruvate kinase activities but significantly increased glucose-6-phosphate dehydrogenase activity in the liver compared to diabetic rats. This study shows that the feeding of the two test diets to diabetic rats results in alterations in the metabolism of glucose with subsequent reduction in plasma glucose concentration.

Cambios en Algunas Enzimas Hepáticas en ratas con Diabétes Inducida Mediante Estreptozotocina, Alimentadas con Extracto de Sapogenina de ñame Amargo *(Dioscorea polygonoides)* o Diosgenina Comercial

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RESUMEN

Se investigaron los efectos del extracto de sapogenina esteroidal del ñame amargo o la diosgenina comercial, sobre los cambios enzimáticos del hígado. A ratas Wistar machos con diabetes les fueron suministradas dietas con suplementos de 1% de extracto de sapogenina esteroidal o diosgenina comercial por espacio de tres semanas. Se evaluaron los niveles de glucosa plasmática y las actividades de la glucosa-6-fosfatasa hepática, el piruvato kinasa y la glucosa-6-fosfato deshidrogenasa. Asimismo fueron medidos el colesterol total del hígado, el colesterol HDL y el fosfolípido total. La glucosa plasmática disminuyó significativamente ($p < 0.05$) en las ratas diabéticas a las que se les alimentó con las tres dietas de la prueba, en comparación con el control diabético. Las tres dietas de la prueba disminuyeron significativamente la actividad de la glucosa-6-fosfatasa en comparación con el control diabético. Las actividades de la ATP-citrato liasa, la piruvato kinasa y la glucosa-6-fosfato deshidrogenasa disminuyeron significativamente en el hígado de las ratas diabéticas, en comparación con el control normal. El suplemento dietético con extracto de sapogenina esteroidal de ñame amargo o la diosgenina comercial no alteró de manera significativa las actividades de la ATP-citrato liasa y la piruvato kinasa, pero aumentó significativamente la actividad de la glucosa-8-fosfato deshidrogenasa en el hígado, en comparación con las ratas diabéticas. Este estudio demuestra que la alimentación con las dos dietas de prueba suministrada a las ratas diabéticas, produce alteraciones en el metabolismo de la glucosa, con la consiguiente reducción de la concentración de glucosa plasmática.

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INTRODUCTION

Diabetes is the most common of the endocrine disorders and poses a serious challenge to healthcare worldwide. The prevalence of the different types of diabetes varies in different parts of the world. However, it is projected that by 2010 at least 239 million people will be affected by the disease (1). In Jamaica, the point prevalence of diabetes mellitus in the 15-year-and-over age group is estimated to be 17.9% (2) while in the United Kingdom it is believed to be 2.4% of the adult population (3).

Diabetes poses a threat to developing as well as developed countries. In the United States of America (USA) for example, diabetes ranks sixth as the primary cause of death and has an estimated annual economic cost of between $85 to $92 billion, two-thirds of which is as a result of lost productivity because of admission to hospital or death (4).

Uncontrolled diabetes may result in the death of the patient or the development of complications. Some of the characteristic long-term complications of diabetes are retinopathy (with potential loss of vision), arteriosclerosis, peripheral vascular and cerebrovascular disease, peripheral neuropathy, development of foot ulcers and amputation.

Diabetes has been shown to decrease the activities of enzymes in the glycolytic and pentose phosphate pathways, while increasing the activities of gluconeogenic, glycogenolytic and lipolytic pathways (5, 6). Several reports show the hypoglycaemic effect of diosgenin in normal and diseased states (7–9) however, the mechanism by which diosgenin reduces blood glucose is not known. The present study was undertaken to determine the effect of bitter yam sapogenin extract on diabetes-induced changes in hepatic enzyme activities associated with glucose and lipid metabolism with a view to elucidating the probable mechanism by which sapogenin extract reduces blood glucose.

METHODS

Sample Preparation: Freshly harvested tubers of bitter yam (*Dioscorea polygonoides*) were obtained from Muirhouse, St Ann, Jamaica. Sapogenin was extracted from Jamaican bitter yam using the method of Morris *et al (*10). Fifty grams of freshly harvested tuber was blended with 50 ml water and 115 ml of 3.5 N HCl was added to the mixture and refluxed for three hours. It was then filtered and washed to neutrality with distilled water. The filter paper and residue were dried at 65°C – 70°C overnight and then extracted with petroleum ether (30–60 BP) for six hours using Soxhlet extraction. The volume was reduced using a rotary evaporator and filtered through a sintered glass funnel. The residue and the sintered glass were heated for 30 minutes at 95°C in an oven and the residue collected for the feeding experiment. The sapogenin extract and 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 Δ^2 diosgenin) or commercial diosgenin/100 g of rat basal diet (9).

Animals and diet: Adult wistar rats (n = 32) obtained from The University of the West Indies (UWI) Animal House, were divided into four groups, by weight, for a three-week study (eight rats per group, mean ± SEM body weight 249.0 ± 0.3 g). The groups were composed as follows: non-diabetic rats receiving normal diet (normal); diabetic rats fed normal diet (diabetic); diabetic rats fed sapogenin extract (1% of normal diet (sapogenin extract)) and diabetic rats fed commercial diosgenin (1% of normal diet (diosgenin)).

Three of the four groups received a single injection of streptozotocin (Sigma, 60 mg/Kg-body weight in 0.05M-citrate buffer, pH 4.5) intraperitoneally. The fourth group, the normal control group, was injected intraperitoneally with an equivalent amount of buffer (0.05M-citrate buffer, pH 4.5). After eight days, blood was obtained from the tail vein and the level of blood glucose determined using the method of Teller (11). Initial blood glucose for normal rats was 4.77 ± 0.75 mmol/L while the initial 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 230 g/kg, fat 45 g/kg, fibre 60 g/kg, ash 80 g/kg and carbohydrate 585 g/kg. Rats were housed in stainless steel cages in a room kept on a 12-hour light-dark cycle, and were allowed to have access to their respective diets and water *ad libitum*. The cages were cleaned daily. Body weight was recorded weekly. The difference between food given and the left over was taken as food intake. The rats were fed on their respective diets for 21 days and sacrificed by decapitation after an overnight fast. Fasting plasma samples were obtained *via* heart puncture for glucose determination. Liver samples were taken for lipid and enzyme assays. Approval for the study was obtained after the presentation of the protocol to the Board of the Department of Basic Medical Sciences, Biochemistry Section, UWI, Mona.

Metabolite assays: Glucose was determined using the method of Teller (11). Total lipid in the liver was extracted by the method of Bligh and Dyer (12). The determination of total cholesterol was by the method of Zlatkis *et al* (13). High-density lipoprotein cholesterol was measured by precipitating the low-density lipoprotein cholesterol with phosphotungstate and magnesium and finding the amount of cholesterol in the supernatant (14). Although this method was originally devised for humans, it was practicable for this study. Total phospholipid was assessed using a colorimetric method (15).

Enzyme assays: Glucose-6-phosphatase activity was measured by the amount of inorganic phosphate released following incubation with glucose-6-phosphate (16). The activities of ATP citrate lyase, glucose-6-phosphate dehydrogenase and pyruvate kinase in the liver were determined by measuring the change in extinction due to NADP reduction or NADH oxidation (6).

Statistical analysis

Data were analyzed by Duncan's Multiple Range Test. Differences between groups were considered significant at $p < 0.05$ (17).

RESULTS

Table 1 shows food intake, body and liver weight and final plasma glucose in diabetic rats fed yam sapogenin extract or commercial diosgenin. The diabetic rats (fed supplemented and unsupplemented diets) lost weight significantly compared to the normal group, although there was no significant difference in their food intake. There was no significant difference in liver weight. The test diets significantly lowered plasma glucose levels compared with diabetic control group.

Table 1: Food intake, body and liver weight and final plasma glucose in diabetic rats fed yam sapogenin extract or commercial diosgenin

Group	Normal extract	Diabetic diosgenin	Yam sapogenin	Commercial
Final body wt (g)	325.4 ± 9.2[a]	247.3 ± 27.0[b]	190.8 ± 17.4[b]	193.3 ± 8.8[b]
Initial body wt (g)	248.4 ± 16.2[a]	249.3 ± 9.2[a]	249.8 ± 14.1[a]	248.4 ± 16.8[a]
Daily food intake per rat (g)	14.6 ± 0.0[a]	14.5 ± 0.1[a]	14.6 ± 0.1[a]	14.4 ± 0.1[a]
Liver wt (g)	7.5 ± 0.4[a]	6.6 ± 0.6[a]	7.2 ± 0.6[a]	6.3 ± 0.4[a]
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 ± SEM. Figures in rows that share different letter superscripts are significantly different among the groups ($p < 0.05$).

Table 2 shows the effect of the dietary supplements on some metabolic enzymes in the liver. The activity of glucose-6-phosphatase was significantly different in the liver of diabetic control rats when compared to the normal group. The activities of ATP-citrate lyase, pyruvate kinase and glucose-6-phosphate dehydrogenase were significantly different in the liver of diabetic rats compared to normal control. Treatment with yam sapogenin extract or commercial diosgenin significantly lowered glucose-6-phosphatase activity when compared to the diabetic control. Supplementation of the diet with bitter yam steroidal sapogenin extract or commercial diosgenin did not result in significant difference in the activities of ATP citrate lyase and pyruvate kinase but there was a significant difference in glucose-6-phosphate dehydrogenase activity in the liver of controls compared to diabetic rats.

Table 3 shows the effect of yam steroidal sapogenin extract or commercial diosgenin on the ratio of HDL-cholesterol and phospholipid to total cholesterol. The ratio of HDL-cholesterol and phospholipid to total cholesterol was significantly lowered in diabetic rats compared to the normal group. Supplementation of the diet with sapogenin extract or commercial diosgenin resulted in a significant difference in their ratios, restoring them to almost those of the normal group.

Table 2: Effect of yam steroidal sapogenin extract and commercial diosgenin on some metabolic enzymes in the liver

Group	Glucose-6-phopshate (Sp Act = µmole/min/mg protein) × 10^{-2}	ATP-citrate kinase	Pyruvate lyase (Sp Act = nmole/min/mg protein	Glucose-6-phosphatase dehydrogenase
Normal	5.4 ± 1.4[a]	5.2 ± 0.1[a]	13.7 ± 3.1[a]	3.0 ± 0.5[a]
Diabetic	11.3 ± 1.4[b]	1.4 ± 0.1[b]	4.1 ± 3.0[b]	0.9 ± 0.2[b]
Yam sapogenin extract	7.6 ± 1.9[a]	1.7 ± 0.2[b]	5.7 ± 0.7[b]	2.1 ± 0.4[a]
Commercial diosgenin	8.3 ± 1.0[a]	1.9 ± 0.2[b]	7.2 ± 0.7[b]	1.6 ± 0.2[a]

Values expressed as mean ± SEM. Figures in columns that share different letter superscripts are significantly different among the groups ($p < 0.05$).

Table 3: Effect of yam steroidal sapogenin extract and commercial diosgenin and the ratio of HDL-cholesterol and phospholipid to total cholesterol

Group	HDL cholesterol to total cholesterol ratio (%)	Total cholesterol: total phospholipid ratio (%)
Normal	45.7[b]	0.13[b]
Diabetic	24.1[a]	0.23[a]
Yam sapogenin extract	61.4[b]	0.14[b]
Commercial diosgenin	52.2[b]	0.16[b]

Values expressed as mean ± SEM. Figures in the same column with different superscripts are statistically significant among the groups ($p > 0.05$).

DISCUSSION

The loss in weight in the diabetic group and those fed sapogenin extract or commercial diosgenin despite similar food consumption in this short term study may be indicative of the food not being transformed into weight gain and this may be due to the concept of the disease as one of starvation in the midst of plenty. Of note, however, is the observance of no significant difference in the liver weight among the groups.

In the diabetic state, there are alterations in the specific activities of several glycolytic, NADPH generating and gluconeogenic enzymes in the liver (18). The liver is insulin dependent and requires insulin for glucose uptake, glucose phosphorylation and the entry of glucose-6-phosphate into the metabolic pathway.

In the present study, the activities of glycolytic and lipogenic enzymes were significantly lowered in the liver of the diabetic rats compared to the normal, while the activities

of gluconeogenic enzymes were increased. Supplementation of the diet with bitter yam steroidal sapogenin extract or commercial diosgenin resulted in an increase in the activity of pyruvate kinase. Pyruvate kinase catalyzes the dephosphorylation of phospho-enolpyruvate to pyruvate. An increase in the activity of pyruvate kinase indicates that more pyruvate is being chanelled into the tricarboxylic cycle pathway where it is converted to citrate. Citrate is formed in the mitochondria by the condensation of acetylCoA and oxaloacetate (19). Citrate then diffuses into the cytosol where it is cleaved by the enzyme ATP citrate lyase for fatty acids and bile acids synthesis. Daikuhara *et al* and Kornacker and Ball (20, 21) reported decrease in the activity of ATP citrate lyase in the diabetic state. The observed increase in the activity of liver ATP citrate lyase of rats fed sapogenin extract from yam is indicative of cleavage of the citrate for bile acids and fatty acids synthesis which is consistent with reports by Yamamoto *et al* (22, 23) that saikosaponins increased hepatic lipogenesis and cholesterogenesis.

Glucose-6-phosphate dehydrogenase catalyzes the conversion of glucose-6-phosphate to 6-phosphogluconate with the generation of NADPH, the reducing power for bile acids synthesis. This enzyme is an important site of control in the pentose phosphate pathway, which provides NADPH for lipogenesis and ribose for the synthesis of nucleic acids. The observed increases in the activities of glucose-6-phosphate dehydrogenase and ATP citrate lyase in the liver of rats fed sapogenin extract from bitter yam are indicative of increased cholesterol and bile acids synthesis which may be geared towards bile acids and cholesterol replenishment (24). The glycolytic cycle provides acetylCoA for the synthesis of long chain fatty acids. The observed increase in the activities of glucose-6-phosphate dehydrogenase and ATP-citrate lyase is indicative of the increased rate of liver lipogenesis in diabetic rats fed steroidal sapogenin.

Glucose-6-phosphatase catalyzes the final step of gluconeogenesis which involves the release of glucose from glucose-6-phosphate. There was an observed increase in the activity of glucose-6-phosphatase in the diabetic controls compared to normal rats (25). Supplementation of the diet with commercial diosgenin and bitter yam steroidal sapogenin extract resulted in a decrease in the activity of glucose-6-phosphatase. This shows decreased glucose synthesis which may have resulted from increased glucose uptake as demonstrated by the increase in pyruvate kinase activity. Increase in liver glucose uptake may be due to alterations in the hepatic membrane cholesterol to phospholipid ratio leading to the partial restoration of glycolysis towards normal with subsequent decrease in gluconeogenesis. This may account for the observed decrease in plasma glucose in the groups fed commercial diosgenin and bitter yam steroidal sapogenin extract. Changes in the cholesterol to phospholipid ratio have been reported to affect the motion of the lipid hydrocarbon chains and physical properties of the bilayer (26).

In conclusion, the feeding of sapogenin extract from bitter yam or commercial diosgenin to diabetic rats result in alterations in the metabolism of glucose with subsequent reduction in plasma glucose concentration. However, this study only provides indirect evaluation of sapogenin extract for humans. Further studies on the toxicity of the extract in animal models are required before trials in humans.

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