INCREASED PLASMA AND LIVER LIPIDS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS: EFFECTS OF YAM (Dioscorea cayenensis) OR DASHEEN (Colocasia esculenta) EXTRACT SUPPLEMENTS

Felix O. Omoruyi, Phillip B. Grindley, Helen N. Asemota, Errol Y. St. A. Morrison

Keywords: cyanoglucoside, lipid, yam, dasheen, diabetes

SUMMARY

The effects of incorporating cyanoglucoside extracts from yam or dasheen into the diet of streptozotocin-induced diabetic Wistar rats were studied for four weeks. Intestinal lipase activity, and plasma glucose and lipid levels were determined. Also, liver total lipids, activities of ATP citrate lyase, pyruvate kinase and 6-phosphogluconate dehydrogenase were assessed. Dasheen extract significantly lowered plasma levels of glucose and triglycerides (p < 0.05). Yam or dasheen extract supplementation significantly decreased plasma levels of cholesterol and HDL cholesterol. Similarly, supplementation with commercial linamarin significantly reduced plasma HDL cholesterol. Yam or dasheen extract supplementation significantly increased the activity of 6-phosphogluconate dehydrogenase. The two supplements significantly lowered liver total lipids towards normal, whereas commercial linamarin supplementation significantly increased liver total lipids above normal. These observations showed the acute intake of dasheen extract to lower the level of triglycerides, which is desirable in diabetes, however, as it also lowered plasma HDL cholesterol, it may not be considered a useful dietary supplement in diabetics.

INTRODUCTION

Yams (Dioscorea spp.) are important staples in the diet of millions of people in tropical and subtropical countries. Mankind have been using yam for food since time immemorial and it is still used extensively throughout the world. The rate of use, however, varies considerably from country to country. Dietary value of yam is based on carbohydrates, proteins, vitamins and minerals, whereas the amount of lipids is very low (1). Most yam species contain large amounts of plant steroids, primarily diosgenin, a sapogenin precursor in the synthesis of progesterone.

Dasheens (Colocasia esculenta) or ‘taro’ are cultivated and used for food in various parts of the tropics and in subtropical regions (2). The corn, an important source of staple starch, is the major economic part of dasheen. Dasheen and cocoyam are described as healing plants with various medicinal properties (3). In Hawaii, the cut raw rootstock is rubbed onto wounds to prevent bleeding, and the cut raw petiole is used as an anesthetic as well as to prevent swelling caused by insect bites and stings. The corn is used to treat indigestion and as a laxative.

Peifer and Guzman (4) performed a study in hypercholesterolemic rats, and showed that diosgenin decreased cholesterol absorption, and increased hepatic cholesterol synthesis and biliary cholesterol secretion. It inhibited cholesterol uptake in an everted gut preparation (5).

The prevalence of diabetes in the Caribbean islands is high, ranging from 15% to 20% of total population (6,7), and yam is one of the staple foods. Cyanoglucosides have been implicated in the aggrava-
tion of diabetes (8,9). Different varieties of yams have been found to contain linamarin as the major cyanoglucoside (10,11). The present study was designed to investigate the effects of consumption of cyanoglucoside extract from yam (*Dioscorea cayenensis*, cv. round leaf yellow yam) on plasma and liver lipid metabolism in streptozotocin-induced diabetic rats. Dasheen is also commonly used in the Caribbean. A similar investigation was performed using dasheen extract for comparison.

**MATERIAL AND METHODS**

Fresh tubers of yam (*Dioscorea cayenensis* cv. round leaf yellow yam) and dasheen (*Colocassia esculenta*) about a week after harvest were purchased from a local urban market in Kingston, Jamaica. They were peeled, sliced and dried at 60 °C to constant weight, then ground to fine powder.

Cyanoglucoside extracts from yam or dasheen were made by treating the ground samples with warm (70-75 °C) 80% ethanol. The level of endogenous cyanoglucoside, linamarin, was evaluated by a modification of the method of Cooke et al. (12), in which the cyanide liberated by enzyme hydrolysis was quantified using alkaline picrate. Ethanol was removed by adding an equivalent amount of cyanoglucoside extracts from the respective tubers. This was based on the report that in some countries, people eat as much as 1.0 kg of yam per day (13). The average weight of a human is estimated at 75 kg, therefore, the amount of cyanoglucoside extract added to the diet in the present study was calculated on the basis of rat weight. The crude extract contained, among other ingredients, 25-30 mg of cyanoglucoside per kilogram of feed.

Wistar rats were obtained from the University of West Indies Animal House (7 rats per group, mean body weight 272.58 g). Five groups of rats were used in the 4-week study, as follows: healthy rats receiving normal diet (normal); diabetic rats fed normal diet plus commercial linamarin (Sigma Chemical Company, St. Louis, MO, USA) (linamarin). Four of the five groups received a single injection of streptozotocin (Sigma; 65 mg/kg body weight in 0.05 M citrate buffer, pH 4.5) intraperitoneally. The fifth, normal control group of rats were injected an equivalent amount of buffer (0.05 M citrate buffer, pH 4.5) intraperitoneally. After 8 days, blood was drawn from the tail and the blood glucose level was determined. Initial blood glucose in normal rats was 3.97±0.42 mmol/L, whereas in diabetic rats and in those administered yam or dasheen extracts or commercial linamarin supplements it ranged from 18.86±4.47 to 27.39±0.18 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 following approximate chemical composition: protein 23%, fat 4.5%, fiber 6.9%, ash 8.0%, and carbohydrate 58.5%.

Rats were housed in stainless steel cages in a room kept on a 12-hour light-dark cycle, and were allowed free access to their respective diet and water. The cages were cleaned daily. Body weight change and total food intake were recorded weekly. The rats were fed the respective diet for 28 days and sacrificed by decapitation after overnight fast.

Fasting blood samples were obtained for glucose determination. Liver samples were taken for enzyme assays. Blood glucose was determined by the colorimetric method of Teller (14), cholesterol by the method of Zlatkis et al. (15), and plasma triglycerides by the method of Gottfried and Rosenberg (16). High-density lipoprotein (HDL) cholesterol was measured by precipitating low-density lipoprotein (LDL) cholesterol with phosphotungstate and magnesium, and determining the amount of cholesterol in the supernatant, according to Lopez-Virella et al. (17). Although this method was originally devised for human plasma, it was practicable for this study. Due consideration was exercised for differences that may arise as the result of variation in the lipid concentration and apoprotein composition between the two species. Total liver lipids were extracted by the method of Bligh and Dyer (18). The intestine of each rat which was free from 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. Lipase activity was measured by titrimetric method quantifying the amount of fatty acids liberated by the action of the enzymes on triolein substrate (19). The activities of ATP citrate lyase and pyruvate kinase were determined in the liver by measuring the change in extinction due to NADH oxidation, and liver 6-phosphoglu-
conate dehydrogenase was assayed by measuring the change in extinction due to NADP⁺ reduction, as described by Storey and Bailey (20).

All data were expressed as means ± SEM. ANOVA was used to test for between group differences. Duncan’s multiple range test was used to test for significance of differences between the means, with p<0.05 as the level of significance (21).

RESULTS

Table 1 shows body and liver weight changes, feed intake, and glucose level in diabetic rats fed yam or dasheen extract. The body weight of diabetic rats was significantly reduced even though there was no significant difference in the feed intake. The greatest weight loss was seen in diabetic rats fed commercial linamarin supplement. Liver weight was significantly reduced in all groups fed the respective supplements relative to healthy control rats.

Table 2 shows the effect of yam or dasheen extract on lipase activity in two segments of rat intestinal mucosa. Only commercial linamarin supplementation significantly lowered the activity of this enzyme below that obtained for healthy control rats.

Figure 1 shows blood lipid profile in diabetic rats fed extract of yam or dasheen. Total cholesterol levels were significantly decreased in rats fed yam or dasheen diet compared to healthy control rats and diabetic rats fed normal or commercial linamarin supplements, respectively. Triglyceride level was significantly higher in the group fed commercial linamarin compared to healthy control group. Dasheen extract significantly lowered the level of triglycerides as compared with healthy control rats or diabetic rats fed normal diet. Similarly, yam or dasheen extract or commercial linamarin significantly reduced HDL cholesterol levels compared to healthy control rats or diabetic rats fed normal diet.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Diabetic</th>
<th>Yam extract treated</th>
<th>Dasheen extract treated</th>
<th>Commercial linamarin treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>272.60±19.10</td>
<td>272.70±15.40</td>
<td>271.00±17.80</td>
<td>272.80±17.00</td>
<td>273.80±24.60</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>281.00±15.05</td>
<td>191.00±12.10*</td>
<td>166.00±6.40**</td>
<td>165.10±6.60**</td>
<td>158.80±17.40**</td>
</tr>
<tr>
<td>Feed intake (g/week)</td>
<td>99.76±3.12</td>
<td>97.37±5.64</td>
<td>96.61±2.45</td>
<td>99.19±3.76</td>
<td>101.96±0.69</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>7.37±0.31</td>
<td>5.98±0.41*</td>
<td>4.83±0.21**</td>
<td>4.81±0.26*</td>
<td>4.93±0.31*</td>
</tr>
<tr>
<td>Final blood glucose (mmol/L)</td>
<td>4.74±0.30</td>
<td>19.50±5.16*</td>
<td>18.72±3.06**</td>
<td>13.18±3.53*</td>
<td>20.87±3.25*</td>
</tr>
</tbody>
</table>

Horizontal * denotes significant differences (p<0.05) from control, whereas # denotes significant differences (p<0.05) from respective diabetic groups fed normal diet; Final blood glucose: * denotes significant differences (p<0.05) from healthy control group, whereas # denotes significant differences (p<0.05) from dasheen extract (Duncan’s Multiple Range Test)

<table>
<thead>
<tr>
<th>Group</th>
<th>Upper</th>
<th>Lower</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>19.70±3.55</td>
<td>16.61±1.69</td>
</tr>
<tr>
<td>Diabetic</td>
<td>14.50±2.14</td>
<td>13.20±1.60</td>
</tr>
<tr>
<td>Yam extract treated</td>
<td>13.35±0.84</td>
<td>12.40±1.06</td>
</tr>
<tr>
<td>Dasheen extract treated</td>
<td>17.35±5.44</td>
<td>16.39±4.91</td>
</tr>
<tr>
<td>Commercial linamarin treated</td>
<td>8.31±0.33*</td>
<td>15.60±4.91</td>
</tr>
</tbody>
</table>

Vertical * denotes significant difference (p<0.05) from control (Duncan’s Multiple Range Test)

Figure 1. Blood lipid profile in diabetic rats fed extract of yam or dasheen

Table 2. Effect of yam or dasheen extract on lipase activity in two segments of rat intestinal mucosa

Table 1. Body and liver weight changes, feed intake, and glucose level in diabetic rats fed yam or dasheen extract

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Diabetic</th>
<th>Yam extract treated</th>
<th>Dasheen extract treated</th>
<th>Commercial linamarin treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
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</tr>
<tr>
<td>Final body weight (g)</td>
<td>281.00±15.05</td>
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<td>158.80±17.40**</td>
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<tr>
<td>Feed intake (g/week)</td>
<td>99.76±3.12</td>
<td>97.37±5.64</td>
<td>96.61±2.45</td>
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</tr>
<tr>
<td>Liver weight (g)</td>
<td>7.37±0.31</td>
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<td>4.83±0.21**</td>
<td>4.81±0.26*</td>
<td>4.93±0.31*</td>
</tr>
<tr>
<td>Final blood glucose (mmol/L)</td>
<td>4.74±0.30</td>
<td>19.50±5.16*</td>
<td>18.72±3.06**</td>
<td>13.18±3.53*</td>
<td>20.87±3.25*</td>
</tr>
</tbody>
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Horizontal * denotes significant differences (p<0.05) from control, whereas # denotes significant differences (p<0.05) from respective diabetic groups fed normal diet; Final blood glucose: * denotes significant differences (p<0.05) from healthy control group, whereas # denotes significant differences (p<0.05) from dasheen extract (Duncan’s Multiple Range Test)
Table 3 shows the effect of yam or dasheen extract supplement on the activities of ATP citrate lyase, pyruvate kinase and 6-phosphogluconate dehydrogenase in the liver. ATP citrate lyase activity in diabetic rats fed normal diet was significantly reduced compared with healthy control group. Diabetic rats fed normal diet significantly lowered the activity of 6-phosphogluconate dehydrogenase compared to healthy control rats. Yam extract or commercial linamarin significantly increased the activity of this enzyme above that recorded in diabetic rats fed normal diet.

Table 3. Effect of yam or dasheen extract supplements on the activities of ATP citrate lyase, pyruvate kinase and 6-P-gluconate dehydrogenase in the liver

<table>
<thead>
<tr>
<th>Group</th>
<th>ATP citrate lyase</th>
<th>Pyruvate kinase</th>
<th>6-P-gluconate dehydrogenase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.84±0.10</td>
<td>2.85±0.14</td>
<td>28.74±2.11</td>
</tr>
<tr>
<td>Diabetic</td>
<td>1.41±0.27*</td>
<td>1.46±0.27*</td>
<td>18.72±1.50*</td>
</tr>
<tr>
<td>Yam extract treated</td>
<td>1.48±0.04*</td>
<td>1.49±0.04*</td>
<td>34.49±6.00**</td>
</tr>
<tr>
<td>Dasheen extract treated</td>
<td>1.41±0.10*</td>
<td>1.44±0.10*</td>
<td>22.58±2.81</td>
</tr>
<tr>
<td>Commercial linamarin treated</td>
<td>1.95±0.46*</td>
<td>1.99±0.46*</td>
<td>34.60±1.33*</td>
</tr>
</tbody>
</table>

Vertical * denotes significant differences (p<0.05) from controls, whereas # denotes significant differences (p<0.05) from diabetic groups fed normal diet and dasheen extract, respectively (Duncan’s Multiple Range Test).

DISCUSSION

Data on the intestinal lipase activity, obtained in this study, suggest that yam extract or commercial linamarin supplementation may interfere with lipid absorption from the gut through nonspecific interaction with intestinal lipase, leading to its partial inhibition. This in turn results in lower levels of absorbable lipids in the blood, which may partly account for the observed decrease in blood lipids in rats fed yam extract or commercial linamarin.

Adamson (22) has reported that dietary fiber in yam lowered serum cholesterol in poultry and rats. The presence of diosgenin in yam may also be a contributory factor in the observed decrease in blood cholesterol (4,23). Earlier study by Anderson and Chen (24) showed selective lowering of plasma total cholesterol while increasing plasma HDL cholesterol by fiber diet. Studies by Bainton et al. (25) showed that triglycerides are independently related to coronary heart disease. The observed significant decrease in plasma triglycerides may be beneficial to diabetics.

The liver is the only organ that can catabolize and excrete quantitatively important amounts of cholesterol. Glomset (26) reports that HDL alters the balance of unesterified cholesterol between plasma and cells. It does this by increasing its utilization in the lecithin cholesterol acyltransferase (LCAT) system to form cholesterol ester. It has also been shown by Badges and Bjorkenid (27) using human arterial tissue, that HDL is the principal carrier of cholesterol from the peripheral tissue to the liver. Marcel et al. (28) have proposed that HDL may decrease the rate of cholesterol entry into the cells via LDL and increase the rate of cholesterol release from the cell, again explaining the possible protective effect of HDL. This study showed a significant decrease in the plasma level of HDL in rats fed supplements of yam or dasheen extract or commercial linamarin. The percentage of plasma HDL cholesterol fraction in total cholesterol was lowest in the groups fed dasheen extract or commercial linamarin. This indicates that acute consumption of dasheen diet may increase the risk of atherosclerosis and coronary heart disease development associated with diabetic condition. This may constitute a major drawback in the consumption of dasheen by diabetics because a shift of cholesterol to HDL cholesterol is a desired goal in the management of diabetes.
The hepatic glycolytic enzyme, pyruvate kinase, did not respond actively as there was no significant change in the activity of this enzyme in rats fed supplemented diets compared with diabetic group of rats. This suggests that the hepatic glycolytic pathway may not play an active role in the observed hypoglycemic property of yam or dasheen extract. This may account for the increase in the activities of 6-phosphogluconate dehydrogenase and ATP citrate lyase, which may be geared towards NADPH and acetyl group generation for lipid synthesis.

In conclusion, the present study showed that acute consumption of dasheen lowered the level of triglycerides, which is desirable in diabetes, however, its property of lowering plasma HDL cholesterol suggested that it may not be a useful dietary supplement for diabetics.

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REFERENCES


