HEPATIC FUNCTION ENZYMES AND LIPID PEROXIDATION IN STREPTOZOTOCIN-INDUCED DIABETIC RATS FED BITTER YAM (DIOSCOREA POLYGONOIDES) STEROIDAL SAPOGENIN EXTRACT

Marie A. McAnuff\textsuperscript{1}, Felix O. Omoruyi\textsuperscript{1}, Errol Y. Morrison\textsuperscript{1}, Helen N. Asemota\textsuperscript{1,2}

SUMMARY

Diabetic male wistar rats (n=32) were fed diets supplemented with 1% bitter yam steroidal sapogenin extract or commercial diosgenin for three weeks. Thiobarbituric acid reactive substances (TBARS), conjugated dienes, lipid profile and the activities of alanine and aspartate transaminases and acid phosphatase were measured in the liver. Diabetic rats (groups B-D) lost weight significantly (p<0.05) compared with the normal group even though there was no significant difference (p<0.05) in their feed intake. There was no significant change (p<0.05) in liver weight. Test diets significantly lowered plasma glucose level. Total cholesterol and VLDL-cholesterol decreased significantly (p<0.05), while HDL-cholesterol levels increased significantly in group C and D rats compared to group B rats. There was no significant change in the levels of phospholipids and triglycerides. The feeding of commercial diosgenin and yam sapogenin extract resulted in a significant decrease in conjugated dienes and TBARS compared with the diabetic group fed normal diet and normal rats. Yam extract and commercial diosgenin also caused a significant decrease in the diabetes-induced increase in the activity of alanine transaminase. However, they had no effect on the aspartate and acid phosphatase levels. The study showed that the feeding of bitter yam steroidal sapogenin extract to diabetic rats may result in alterations in the lipid composition of the liver with subsequent reduction in lipid peroxidation. Data from this study also showed improvement in liver damage associated with diabetes.

INTRODUCTION

Diabetes is by far the most common of endocrine disorders and a major threat to health care worldwide. It is projected that by 2010, at least 239 million people will be affected by the disease (1). Diabetes poses a threat to developing as well as developed countries. In America, for example, diabetes ranks sixth as the primary cause of death, and has an estimated economic cost of 85 to 92 billion USD, two-thirds of which are the result of productivity losses because of admission to hospital or death (2). The prevalence of diabetes is high in the Caribbean; in Jamaica, for example, the point prevalence of diabetes mellitus in the \textless 15 age group is estimated to be 17.9\% (3).

Oxidative stress is thought to be a major contributor to cardiovascular disease in diabetes mellitus (4). In fact, it is well documented that diabetes is associated with increased oxidative stress, as evidenced by the increased accumulation of lipid peroxides in the plasma of rats (5) and humans (6) with diabetes mellitus.

Several species of wild yams are known to be biologically active against hypertension, arthritis, diabetes mellitus and other physical ailments (7), and...
have been used in traditional medicine in Africa, among the Chinese and other Asiatic people. The saponins or sapogenins present may be the active principles in these tubers (8). The Jamaican wild yam (Dioscorea polygonoides) is used in the preparation of root tonic and contains high levels of sapogenin/saponin. The purpose of the present studies, therefore, was to examine the effects of yam steroidal sapogenin extract on liver lipid peroxidation and function enzymes in streptozotocin-induced diabetic rats.

**MATERIAL AND METHODS**

**Sample preparation**

Samples of wild yam were obtained from Muirhouse, in the Parish of St. Ann, Jamaica. The sapogenins were extracted using the method of Morris et al. (9). The sapogenin extract from bitter yam contains 80% diosgenin, the remaining 20% being made up of β-sitosterol, penogenin, stigmasterol and Δ² diosgenin.

**Animals and diets**

Male wistar rats were obtained from the Animal House, University of the West Indies. The rats were divided into four groups of eight rats each (average weight 248.98 g) as follows: healthy rats receiving normal diet (group A); diabetic rats receiving normal diet (group B); diabetic rats receiving 1% bitter yam sapogenin extract (group C); and diabetic rats fed 1% commercial diosgenin (Sigma Chemical Co., St. Louis, MO, USA) (group D). The normal diet (PMI Feed Inc. Lab diet #5001) was a commercial laboratory diet recommended for rats, hamsters and mice with the nutrient composition of 23% protein, 4.5% fat, 6.0% fiber, 8.0% ash and 58.5% carbohydrate.

Groups B-E received a single injection of streptozotocin (Sigma Aldrich, 65 mg/kg body weight in 0.05 M citrate buffer, pH 4.5), while group A received a single injection of an equivalent amount of buffer. After 8 days, blood was collected from the tail and the level of blood glucose was determined. Rats were considered diabetic if their blood glucose level was four times in excess of the normal.

The cages were cleaned daily and total feed intake was recorded daily. The rats were kept on their respective diet for 21 days and killed after an overnight fast by decapitation. The facilities met the requirements of the Institutional Animal Care and Use Committee.

Fasting plasma and liver samples were obtained for further analyses.

**Lipid peroxidation assays.** Liver lipid peroxidation was determined using the procedure of Tappel and Zalkin (10). Lipid peroxidation was assessed as the amount of thiobarbituric acid reactive substances (TBARS) produced.

**Conjugate diene analysis.** The liver conjugated dienes were determined by the method of Hu et al. (11). Liver lipids were extracted using the method of Bligh and Dyer (12), the extract was dissolved in cyclohexane, and conjugated dienes were measured at 233 nm using an extinction coefficient of 27,000 (mol/L)-¹cm⁻¹.

**Lipid composition analyses.** Liver lipids were extracted with chloroform/methanol (2:1) according to the method of Bligh and Dyer (12). Total cholesterol was determined according to Zlatkis et al. (13), whereas HDL cholesterol was determined using the method of Lopes-Virella et al. (14). Triglycerides were assayed according to Gottfried and Rosenberg (15). Phospholipids were determined using the method of Fiske and Subbarow (16) after digesting with perchloric acid.

**Plasma glucose determination.** Glucose was determined using the method of Teller (17).

**Transaminase assay.** The activities of alanine and aspartate transaminases were determined using the method of Reitman and Frankel (18) as reported by Bergmeyer and Erlich (19).

**Acid phosphatase assay.** The activity of acid phosphatase was determined using the method of Calzyme Laboratories (20).

**Statistical analysis**

Data were analyzed statistically by Duncan’s Multiple Range Test. Differences between groups were considered significant at p<0.05 (21).
RESULTS

Table 1 shows food intake, body and liver weight changes, and final blood glucose in diabetic rats fed yam sapogenin extract or commercial diosgenin. The diabetic rats (fed supplemented and un-supplemented diets) lost weight significantly compared with the normal group, although there was no significant difference in their food intake. The decrease in body weight was more significant in rats fed sapogenin extract or commercial diosgenin. There was no significant change in liver weight. Blood glucose was significantly elevated in diabetic rats compared with the normal controls. Supplementation of diets with bitter yam sapogenin extract and commercial diosgenin significantly decreased blood glucose towards normal.

The liver cholesterol level is shown in Table 2. The feeding of supplemented diets to diabetic rats resulted in a significant decrease in total cholesterol and VLDL-cholesterol levels, and a significant increase in HDL-cholesterol levels.

Table 1. Feed intake, body and liver weight (g) changes in diabetic rats fed yam sapogenin extract or commercial diosgenin

<table>
<thead>
<tr>
<th></th>
<th>Normal control</th>
<th>Diabetic control</th>
<th>Sapogenin extract</th>
<th>Commercial diosgenin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body weight</td>
<td>325.4 ± 9.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>247.3 ± 27.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>190.8 ± 17.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>193.3 ± 8.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Initial body weight</td>
<td>248.4 ± 16.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>249.3 ± 9.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>249.8 ± 14.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>248.4 ± 16.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Daily feed intake (per rat)</td>
<td>14.6 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.5 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.6 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.4 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Blood glucose after feeding (mMol)</td>
<td>5.87 ± 0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.60 ± 2.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.20 ± 0.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.72 ± 0.77&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Liver weight</td>
<td>7.5 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.6 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.2 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.3 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± SEM; values in the same row with different superscript are statistically significant (p<0.05)

Table 2. Influence of yam steroidal sapogenin extract and commercial diosgenin on liver cholesterol levels

<table>
<thead>
<tr>
<th></th>
<th>Total cholesterol (µmol/g liver)</th>
<th>HDL-cholesterol (µmol/g liver)</th>
<th>VLDL + LDL (µmol/g liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>8.06 ± 0.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.15 ± 0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.81 ± 0.78&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic</td>
<td>13.99 ± 0.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.37 ± 0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.25 ± 0.78&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sapogenin extract</td>
<td>11.40 ± 0.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.70 ± 0.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.8 ± 0.58&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Commercial diosgenin</td>
<td>11.92 ± 1.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.22 ± 0.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.85 ± 0.26&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± SEM; values in the same row with different superscript are statistically significant (p>0.05)

Figure 1 shows the effect of yam steroidal sapogenin extract and commercial diosgenin on liver triglyceride and phospholipid levels. Triglyceride and phospholipid concentrations did not differ in normal and diabetic rats. These variables were not significantly affected by the dietary supplements.

Table 3 shows the effect of yam steroidal sapogenin extract and commercial diosgenin on the HDL-cholesterol and phospholipid to total cholesterol ratio. The ratio of HDL-cholesterol and phospholipids to total cholesterol was significantly lowered in diabetic rats compared with the normal group. Supplementation of the diet with sapogenin extract and commercial diosgenin resulted in a significant increase in their ratios, restoring them almost to that of the normal group.
Table 4 shows the amount of lipid peroxidation products in the liver. Bitter yam sapogenin extract and diosgenin had significant effects on the hepatic levels of oxidation products. Diabetic rats receiving the unsupplemented diet had significantly (p<0.05) higher levels of conjugated dienes (Table 4) and TBARS than the supplemented groups. Diabetic rats also had significantly higher levels of conjugated dienes than normal rats.

Table 4. Lipid peroxidation products (TBARS and conjugated dienes) in the livers of rats fed bitter yam sapogenin extract or commercial diosgenin

<table>
<thead>
<tr>
<th></th>
<th>Conjugated dienes (µmole/g liver)</th>
<th>TBARS (nmol/g liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.495 ± 0.040 b</td>
<td>26.1 ± 2.9 b</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0.582 ± 0.068 a</td>
<td>31.3 ± 2.2 b</td>
</tr>
<tr>
<td>Sapogenin extract</td>
<td>0.416 ± 0.023 a</td>
<td>17.7 ± 1.1 a</td>
</tr>
<tr>
<td>Commercial diosgenin</td>
<td>0.428 ± 0.082 a</td>
<td>18.1 ± 2.1 a</td>
</tr>
</tbody>
</table>

Values are means ± SEM; figures in the same row with different superscript are statistically significant (p>0.05)

Table 5 shows the effect of bitter yam steroidal sapogenin extract and commercial diosgenin on the ratio of HDL-cholesterol and phospholipids to total cholesterol

<table>
<thead>
<tr>
<th></th>
<th>HDL cholesterol to total cholesterol ratio (%)</th>
<th>Molar cholesterol: molar phospholipid ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>45.7 ± 2.1 b</td>
<td>0.13 b</td>
</tr>
<tr>
<td>Diabetic</td>
<td>24.1 ± 1.8 a</td>
<td>0.23 a</td>
</tr>
<tr>
<td>Sapogenin extract</td>
<td>61.4 ± 2.8 b</td>
<td>0.14 b</td>
</tr>
<tr>
<td>Commercial diosgenin</td>
<td>52.2 ± 2.3 b</td>
<td>0.16 b</td>
</tr>
</tbody>
</table>

Values are means ± SEM; values in the same row with different superscript are statistically significant (p>0.05)

DISCUSSION

The feeding of 1% diosgenin to hypercholesterolemic rats has been shown to significantly lower plasma and liver lipids (22). In this study, the short-term feeding of bitter yam steroidal sapogenin extract resulted in significantly decreased liver total cholesterol and VLDL cholesterol levels, and significantly increased HDL-cholesterol level (22,23). The increase in HDL-cholesterol levels may be beneficial owing to the negative correlation between HDL-cholesterol levels and cardiovascular diseases. The steroidal sapogenin extract was more effective in decreasing liver lipid distribution than commercial diosgenin. This could be due to the presence of other hypolipidemic agents such as β-sitosterol (24) present in the bitter yam steroidal sapogenin extract.

There was no significant change in triglyceride and phospholipid levels between diabetic and normal groups (25). The cholesterol to phospholipid ratio was significantly increased in the diabetic group compared with normal rats. This may be due to the increased cholesterol levels observed in the diabetic group and the lack of change in the phospholipid levels. Increased cholesterol to phospholipid ratio may affect the motion of the lipid hydrocarbon chains and physical properties of the bilayer (26). The observed hypocholesterolemic properties of yam steroidal sapogenin extract and commercial diosgenin significantly decreased the alanine transaminase activity. Neither the food supplements nor the induction of diabetes had any effect on the activity of acid phosphatase.
cholesterol to phospholipid ratio towards that of normal rats. Ulloa and Nervi (27) have reported that steroid molecules may modify the cholesterol-phospholipid interactions in the membrane by interacting with the hydrophobic part of the membrane. This modification may be beneficial as increases in the levels of cholesterol render membranes more lipophilic and therefore less easy to degrade (28).

Conjugated dienes absorb ultraviolet light at 230–235 nm and are considered by researchers appropriate for the measurement of lipid peroxidation (29). The levels of conjugated dienes and TBARS in the liver measure its susceptibility to lipid peroxidation as well as the antioxidant capability of dietary supplements. In this study, the highest levels of TBARS and conjugated dienes were seen in the diabetic group, suggesting them to be more susceptible to lipid peroxidation compared with normal rats. This result is consistent with numerous reports of increased oxidative stress in the tissues of diabetic rats (30-35). Supplementation of the diet with bitter yam steroidal sapogenin extract or commercial diosgenin reduced the diabetes-induced increase in the liver lipid peroxide levels, and thus reduced the susceptibility of the liver to lipid peroxidation. The effect of bitter yam steroidal sapogenin extract on the activities of the antioxidant enzymes (e.g., superoxide dismutase, glutathione peroxidase, and catalase) is not known. However, these enzymes are involved in the scavenging/inactivation of the reactive oxygen species or redox metal ions before lipid peroxidation takes place, and their assessment in relation to this dietary supplement may elucidate the probable mechanism of action in reducing lipid peroxidation as observed in this study (36).

Recent publications have shown that saponins extracted from soybeans inhibited the formation of lipid peroxides in corn oil samples, owing to their ability to scavenge free radicals (36). The antioxidative activity of the sapogenin extract and commercial diosgenin may also be due to the presence of the hydroxyl groups similar to that reported for sesamol and sesamolinol (37). The sapogenin extract used in this study consisted of β-sitosterol, stigmasterol pennogenin, and diosgenin compounds, which contain more hydroxyl groups compared with the commercial diosgenin, and this may have accounted for the higher antioxidant activity in the sapogenin extract group.

Alanine and aspartate transaminase activities are used as an indicator of hepatocyte damage (38). Acid phosphatase activity is normally high in diseased states and is often used as a tool in clinical investigations (39). Data from this study show that alanine transaminase activity is elevated in diabetes, while the feeding of the sapogenin extract and commercial diosgenin resulted in a significant decrease in alanine transaminase activity. These dietary supplements may protect liver cells from free radical damage.

In conclusion, the study has demonstrated that the feeding of yam sapogenin extract and commercial diosgenin to diabetic rats may lead to alterations in the lipid composition of the liver and reduction in lipid peroxidation, and may prevent liver damage associated with diabetes.

REFERENCES


Acknowledgment. The authors wish to thank Graduate Studies and Research, University of the West Indies, Mona Campus, for providing financial support.