EFFECTS OF DISCOREA DUMENTORUM TUBER SUPPLEMENTED DIET ON PLASMA LIPID PROFILE AND GLUCOSE LEVEL OF HYPERCHOLESTEROLEMIC RATS

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ABSTRACT

This study was designed to investigate the effects of Discorea dumentorum tuber supplemented diet on the plasma lipid profile and glucose level of hypercholesterolemic rats. Thirty two male albino rats (Rattus novergicus) were divided into two groups control and Group1. Rats in control received cholesterol-free diet. Group 1 (hyper control) fed 1% cholesterol + 20% fat diet. On establishing hypercholesterolemia, Group1 rats were sub-divided into three groups (1,2,3), with seven rats in each. Groups 2 and 3 diets were supplemented with white bitter yam and yellow bitter yam flour respectively, while Group1 rats were maintained on the hypercholesterolemic diet. They were fed for four weeks, after which the blood samples were collected via cardiac puncture. Plasma lipid profile (Triglyceride (TG), High density lipoprotein (HDL), Low density lipoprotein (LDL), Total cholesterol (TC) and Glucose level were determined. The result showed significantly higher (p≤ 0.05) levels of plasma TC, LDL, nonsignificant increase in TG, HDL remained unchanged and LDL/HDL ratio was higher in the hyper control rats than the control (p≤ 0.05) while feeding with white and yellow bitter yam (Discorea dumentorum) tuber supplement caused a decrease in the levels of TC, LDL and LDL/HDL ratio when compared with the hypercontrol group (p≤ 0.05). White Discorea dumentorum tuber supplement caused nonsignificant increase level of TG and nonsignificant decrease level of HDL in group2 when compared with the hypercontrol group (p≤ 0.05). Yellow Discorea dumentorum tuber supplement caused a significant increase in the level of TG and significant decrease (p≤ 0.05) in the level of HDL in group3 when compared with the hypercontrol group. Glucose level in hypercontrol rats i.e (group1) was significantly higher compared with the control and on
supplementing the diet with white and yellow *D. dumentorum* a significant reduction in the glucose level was observed when compared with the hypercontrol. It can be concluded that white and yellow *Discorea dumentorum* tuber supplement appears to have hypocholesterolemic and hypoglycemic effects on rats.

**KEYWORDS:** Hypercholesterolemia, *Discorea dumentorum*, lipid profile and glucose level.

**INTRODUCTION**

Cholesterol is a type of fat that is essential for many metabolic processes, a lipid molecule and is biosynthesized by all animal cells because it is an essential structural component of animal cell membranes that is required to maintain both membrane structural integrity and fluidity. Cholesterol enables animal cells to (a) not need a cell wall (like plants and bacteria) to protect membrane integrity/cell-viability and thus be able to (b) change shape and (c) move about (unlike bacteria and plant cells which are restricted by their cell walls). In addition to its importance within cells, cholesterol also serves as a precursor for the biosynthesis of steroid hormones, bile acids and vitamin D. Cholesterol is required to build and maintain membranes; it modulates membrane fluidity over the range of physiological temperatures.[1]

Hypercholesterolemia is a major risk factor for cardiovascular diseases such as atherosclerosis, myocardial infraction, heart attacks and cerebrovascular diseases.[2] The increased blood levels of total cholesterol, Low Density Lipoprotein Cholesterol (LDL-C) and low levels of High Density Lipoprotein Cholesterol (HDLC) has been identified in development of hypercholesterolemia.[3] Diet-induced hypercholesterolemia has long been useful for the assessment of agents interfering with the absorption, degradation and excretion of cholesterol, rather than interfering with cholesterol biosynthesis. Inducing hypercholesterolemia in rats is often through a high fat, high cholesterol diet, with the fat source varying from lard to canola, coconut, soybean, groundnut oil or palm oil. Commercial rations supplemented with cholesterol have also been used for these investigations.[4]

In Nigeria, there seems to be a gradual shift from traditional foods consisting mainly of roots, cereals beans, tubers and vegetables to fatty foods, snacks and drinks which is evident by the increased number of eateries in our society. These changes in dietary pattern toward a more westernized lifestyle could precipitate hyperlipidemia. Plants were the major source of materials used to combat different kinds of ailments in ancient times and quite a number of these plants are being explored in the treatment of various diseases.[5] Many indigenous foods...
in Nigeria have been used for management of hyperglycaemia in the traditional healing system. Many of these foods have become neglected and are almost at the point of extinction. This work is an attempt to revert this trend by identifying and scientifically corroborating the potentials of these foods on health and diseases. One of such is Dioscorea dumentorum (Kunth) Pax (Dioscoreacea). D. dumentorum is a tuber with fleshy edible parts which can be yellow or white.\(^6\) The tuber is commonly called cluster yam (because it occurs naturally in clusters), bitter yam or trifoliate yam, and it is called “ona” by the Igbos of Southeast Nigeria.\(^7\) D. dumentorum is boiled and eaten as a snack in Southeast and southwest Nigeria. Its extract is used for the treatment of diabetes mellitus in traditional medicine.\(^8\) The tubers are also reported to be rich in fiber and contain an alkaloid, dioscorentine, which possesses hypoglycemic activity.\(^9\)

The aim of this study therefore is to assess the effect of Dioscorea dumentorum tuber supplemented diet on the plasma lipid profile of hypercholesterolemic rats.

MATERIALS AND METHODS

Two types of bitter yam (Dioscorea dumentorum) i.e white (A) and yellow (B) types were collected at Ifaki, Ekiti State. Identified in the Harbarium unit of the plant science department of Ekiti State University Ado-Ekiti. It was boiled for about 1-1\(\frac{1}{2}\) hours. The bark was removed and sliced into pieces. After slicing into pieces, the samples was sundried until the mass was constant and ground into powdered form with a blender. The samples A and B was then kept in air tight container until required for diet composition. All chemicals used for the study were of analytical grade (ANALAR).

Animal Groupings

Thirty two (32) male white albino rats (Rattus norvergicus) with average weight of about 100g were used for the study. They were obtained from the Department of Biochemistry, University of Ilorin, Kwara –State, Nigeria. The rats were kept in good conditions and were given normal rat feed and water ad libitum. They were ramdomly divided into four experimental groups (control, group1, group2 and group3). Normal control were fed with standard commercial diet, group 1 were fed modified diet containing 20% fat and 1% cholesterol (Table 1) and after establishing hypercholesterolemia, this group was subdivided into Groups 2 and 3. Group 1 was still maintained on the hypercholesterolemic diet. In addition, Groups 2 and 3 were fed supplemented white and yellow Dioscorea dumentorum tuber respectively while the experimental period lasted for four weeks.
Preparation of Plasma
After an overnight fast, blood samples were collected into lithium-herparin bottles from the rats under chloroform anaesthesia via cardiac puncture. The blood samples were centrifuged at 3000rpm for ten minutes. The clean plasma was collected and kept refrigerated until required for analysis.

Table 1: Diet Composition (g/kg).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn Starch</td>
<td>570</td>
<td>410</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Protein</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Vitamin/mineral mixture</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Cellulose</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>50</td>
<td>200</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>White bitter yam</td>
<td>-</td>
<td>-</td>
<td>570</td>
<td>-</td>
</tr>
<tr>
<td>Yellow bitter yam</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>570</td>
</tr>
</tbody>
</table>

Group 1- Hyper control group.
Group 2- Test group1 (Rats fed white *D. dumentorum* tuber supplemented diet).
Group 3- Test group2 (Rats fed yellow *D. dumentorum* tuber supplemented diet).

Biochemical Analysis
Plasma was analyzed for the following biochemical parameters: Total Cholesterol (TC), HDL-cholesterol and Triacylglycerol (TG) by the method of.[10] Calculation of LDL-cholesterol involves an equation developed by.[11] Commercial kits of the company Randox Laboratories Limited located at Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom were used to determine total cholesterol, triglycerides and high density lipoprotein levels in albino rats by spectrophotometer. Fine test kit of the Company infopia Limited, Korea was used for blood glucose determination.

Total cholesterol test
Procedure
A blank was prepared consisting of 1ml of R1 reagent (4-aminoantipyrine, phenol, peroxidise, cholesterol esterase, cholesterol oxidase, and pipes buffer) with 10µl of distilled water. For the standard, 10µl of the standard cholesterol solution and 1ml of R1 was prepared into clean test tubes. For the test, 10µl of the sample plasma and 1ml of R1 was pipetted into clean tube labelled sample. The tubes were shaken to mix the solution properly and were
incubated at 37°C for 5 minutes. After incubation, the absorbance of the standard and tests were read against the blank at wavelength 546nm. The concentration of cholesterol in the sample was calculated thus.

\[
\text{concentration of cholesterol (mmol/l)} = \frac{\text{absorbance of sample}}{\text{absorbance of standard}} \times \text{concentration of standard}
\]

**Triglycerides test**

**Procedure**

Enzyme reagent (4-aminophenazone, Lipases, Glycerol kinase, Glycerol 3-phosphate oxidase, peroxidase) was reconstituted with 15ml of buffer to form the working reagent. 1ml of the working reagent was pipetted in labelled tubes of blank, standard and samples. 10µl of the standard solution and 10µl of the samples was pipetted into the standard and samples test tubes respectively. All the test tubes were incubated at 37°C for five minutes after which the absorbance of the standard and the samples were read against the blank at wave length 546nm using a spectrophotometer.

\[
\text{concentration of triglycerides (mmol/l)} = \frac{\text{absorbance of sample}}{\text{absorbance of standard}} \times \text{concentration of standard}
\]

**High density lipoprotein test**

**Procedure**

500µl of R1 (Phosphotungstic acid and magnesium chloride) was dispensed into test tubes labelled blank, standard and samples. 200µl of standard cholesterol and 200µl of samples plasma was pipetted into the standard and samples test tubes respectively. The tubes were centrifuged at 2500rpm for five minutes. After centrifugation, 200µl of the supernatant were dispensed into another set of labelled tubes accordingly after which 2ml of cholesterol reagent was added. They were incubated for ten minutes at 37°C. The absorbance of the sample and standard were determined against the blank at 546nm using a spectrophotometer.

\[
\text{concentration of HDL in sample (mmol/l)} = \frac{\text{absorbance of sample}}{\text{absorbance of standard}} \times \text{concentration of standard}
\]

**Low density lipoprotein**

The amount of LDL-cholesterol was calculated using the results of a standard lipid profile which consist of total cholesterol, high density lipoprotein and triglycerides using the formular shown below.
Method for determination of blood glucose level

Fine test (glucometer) test strip for quantitative glucose level was used. Blood was collected from the rats before and after supplementation with white and yellow *D. dumentorum* tuber through tail puncture. It was placed on the test strip and slotted in the fine test glucometer, blood glucose level was read on the glucometer. Glucose was determined after enzymatic oxidation in the presence of glucose oxidase. In each test strip, there is an enzyme called glucose oxidase. This enzyme reacts with the glucose in the blood sample and forms gluconic acid. Gluconic acid reacts with another chemical in the testing strip called ferricyanide. Ferricyanide and gluconic acid reacts to form ferrocyanide. Once ferrocyanide has been formed, the device runs an electric current through the blood sample on the strip. The current is then able to read the ferrocyanide and determine how much glucose is in the sample of blood on the testing strip. A number was displayed on the screen of the glucose testing meter.

Statistical analysis

Analysis of variance was used to test for differences in the groups. All the values were expressed as mean ± standard deviation (SD). Differences were considered to be statistically significant at P≤0.05.

RESULTS

Table 2: Plasma lipid profile after three weeks of feeding hypercholesterolemic diet.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Group 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>1.90±0.00(^a)</td>
<td>3.14±0.70(^b)</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.70±0.10(^a)</td>
<td>0.75±0.05(^a)</td>
</tr>
<tr>
<td>High density lipoprotein (mmol/l)</td>
<td>1.00±0.30(^a)</td>
<td>1.00±0.20(^a)</td>
</tr>
<tr>
<td>Low density lipoprotein (mmol/l)</td>
<td>0.60±0.30(^a)</td>
<td>1.82±0.36(^b)</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>0.60</td>
<td>1.82</td>
</tr>
</tbody>
</table>

Results are expressed as mean±SD; values in the same row with different superscript are significantly different at p≤0.05.
Table 3: Plasma lipid profile after Four weeks of supplementing with white and yellow *D. dumentorum* tuber diet.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>2.03±0.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.67±0.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.68±0.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.93±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.80±0.13&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.61±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.84±0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.99±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>High density lipoprotein (mmol/l)</td>
<td>1.09±0.15&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.59±0.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.85±0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.71±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Low density lipoprotein (mmol/l)</td>
<td>0.58±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.80±0.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.45±0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.77±0.46&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>0.53</td>
<td>1.13</td>
<td>0.52</td>
<td>1.08</td>
</tr>
</tbody>
</table>

Results are expressed as mean±SD; values in the same row with different superscript are significantly different at p≤ 0.05.

Fig. 1: Graph showing glucose Level determination before and after supplementation with white and yellow *D. dumentorum* tuber diet.

**DISCUSSION**

The increase observed in total cholesterol, Triglycerides, LDL and LDL/HDL ratios of the hypercholesterolemic group1 rats compared to the control indicate impaired lipid metabolism in the cholesterol fed rats. It has been reported that excessive dietary intake of fat cause plasma cholesterol to rise by down regulating LDL receptor synthesis as a result of which the uptake of LDL-C via LDL receptor is reduced which results in an increase blood cholesterol level.<sup>[12]</sup> Cholesterol is a soft, waxy substance found among the lipids (fats) in the bloodstream and in all animal body's cells. It is an important part of a healthy body because it is used to form cell membranes, some hormones and is needed for other functions. Alterations in the concentration of major lipids can give useful information on the lipid metabolism and predisposition of an animal to atherosclerosis.<sup>[13]</sup> The reduction in total cholesterol and LDL concentration on supplementation with white and yellow *D.
D. dumentorum tuber suggests the hypocholesterolemic potential of the plant. Lipid lowering effect of *Discorea dumentorum* could be due to inhibition of hepatic cholesterol biosynthesis, increased fecal bile acid secretion and stimulation of receptor-mediated catabolism of LDL-cholesterol and increase in uptake of LDL from blood by liver.\[^{14}\] Similarly, *Discorea dumentorum-* lowered the lipid components in the serum of hyper-cholesterolemic rats, probably by reducing the activity of 3-hydroxy-3-methyl-glutaryl- CoA reductase. Also, the LDL/HDL ratio which is thought to be the atherogenic index of lipoproteins was lower in rats fed with supplemented *D. dumentorum* tuber than in the hypercholesterolemic rats. Although in the past, an increase in the serum total cholesterol level is associated with increased risk of atherosclerosis, however, recent reports indicated that the LDL/HDL ratio is a stronger index of atherogenicity of the lipoproteins rather than individual lipoprotein fraction i.e. the lower the ratio the less atherogenic the lipoprotein profile is thought to be.\[^{15}\] The non significant difference (P ≤ 0.05) between the triglyceride level in the plasma of group1 and group2 is an indication that white *Discorea dumentorum* tuber does not increase blood triglycerides. This suggests that it is unlikely to pose health hazard as high triglyceride level increases the risk of cardiovascular disease. This study is similar with the findings reported by.\[^{16,17}\] in which total cholesterol, triglyceride, low density lipoprotein, LDL/HDL ratio were high in group fed cholesterol diet and the concentration of high density lipoprotein was unaffected. However, the higher triglyceride level was attributed to the inhibition of triglyceride degradation, due to direct inhibitory effect on lipoprotein lipase bound to capillary endothelium. Lipoprotein lipase is vital in the metabolism of triglycerides and is involved in several pathological disorders, including atherosclerosis and obesity.

Hypercholesterolemia has been known through several studies to disturb the oxidant - pro-oxidant balance in favour of prooxidation hence weakening the efficiency of the antioxidant defense system, resulting in ineffective scavenging of free radicals which leads to tissue damage often associated with the development and progress of atherogenesis.\[^{18}\] However, it was reported that the presence of biological antioxidants in plants, which can prevent the uncontrolled formation of free radicals and activated oxygen species and or inhibit their reaction with biological structures may in part be responsible for the beneficial effects of the tuber of *Discorea dumentorum* supplement in hypercholesterolemic rats.\[^{19}\] It was also reported that *Psylliumovate supplemented diet* reduced total cholesterol and LDL-cholesterol in animals and in man.\[^{20}\] Previous work revealed that phytochemical screening of the *Dioscorea dumetorum* showed the presence of saponins, flavonoids and cardiac glycosides in...
addition to alkaloids. Steroid and sapogenin have also been identified in the yam tuber extract but the presence of these chemical agents was not investigated in this study. The hypocholesterolemic effects of *Discorea dumentorum*, observed in the hypercholesterolemic treated rats, may be due in part to the presence of antinutrients. Anti-nutrients commonly found in plant foods such as phytic acid, lectins, phenolic compounds, amylase inhibitors, and saponins have been reported to reduce blood glucose and insulin responses to starchy foods and/or reduce plasma cholesterol and triglycerides. Saponin is known to possess blood cholesterol-lowering activity. The cholesterol-lowering mechanism proposed for saponin is that, it binds cholesterol in the intestinal lumen, and so, cholesterol is less readily reabsorbed. In addition, it may also bind with bile acids, causing a reduction in its enterohepatic circulation, increasing its faecal excretion. Increased bile acid excretion is offset by enhanced synthesis from cholesterol in the liver and consequent lowering of the plasma cholesterol.

The major reason for the marked increase (P ≤ 0.05) in serum glucose in the cholesterol fed rats was uncertain but it could be that the high serum cholesterol decreases the level of glucagon-like peptide-1 which prevent insulin secretion from the pancreatic beta-cells leading to hyperglycemia. The significantly decrease (P ≤ 0.05) observed in fasting glucose concentration in groups 2 and 3 when compared with hypercontrol suggests that supplemented white and yellow *Discorea dumentorum* tuber have low glycemic index in healthy and hyperglycemic humans and are able to reduce blood glucose concentration in hyperglycemic rats. An alkaloid present in the yam extract, dioscoretine, has been reported to possess hypoglycaemic effect. Other alkaloids reported to be present in yam tuber include dihydrosiculoscorine, dioscorine and dumetorine. These alkaloids inhibit alpha-glucosidase and decrease glucose transport through the intestinal epithelium. Besides alkaloids, the tuber contains small quantities of a sapogenin, diosgenin which stimulates the release of insulin and blocks the formation of glucose in the blood stream. It was also reported that lentil or diets containing lentil have low glycemic index in healthy and hyperglycemic humans and are able to reduce blood glucose concentration in hyperglycaemic rats.

**CONCLUSION**

The present research work reveals that the supplementation of *Discorea dumentorum* tuber showed hypoglycemic and hypocholesterolemic effects in hypercholesterolemic rats.
This suggests that *Discorea dumentorum* tuber which forms part of the population’s diet in the past, could be used to lower glucose and cholesterol level in treatment of hyperglycemia and hypercholesterolemia. However, further work on the tuber would be carried out in future studies to establish the mechanism of the action of its hypoglycemic activity.

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