Tropical Journal of Pharmaceutical Research, June 2003; 2 (1): 183-189

Available online at http://www.tjpr.freehosting.net

Research Article

Control of hyperlipidaemia, hypercholesterolaemia and hyperketonaemia by aqueous extract of *Dioscorea dumetorum* tuber

Rachel Nimenibo–Uadia
Department of Biochemistry, Faculty of Science, University of Benin, P.M.B. 1154, Benin City, Nigeria

Abstract

Purpose: *Dioscorea dumetorum (Pax) used in traditional medicine for the treatment of diabetes mellitus possesses hypoglycaemic effect. The present study investigates the effect of oral administration of the aqueous extract of the tuber on blood lipid and ketone levels in alloxan–induced diabetic rats.*
Method: *Wistar strain albino rats were made diabetic with the intraperitoneal administration of alloxan monohydrate. Consequently, an aqueous extract of Dioscorea dumetorum tuber was administered orally to the diabetic rats and their plasma and urine glucose, triacylglycerol, cholesterol and â-hydroxybutyrate concentrations were estimated using standard procedures. Results were compared with untreated normal and diabetic control rats. Phytochemical screening of the extract of the tuber was also carried out.*
Results: *Treatment with the extract significantly ($p<0.05$) reduced elevated blood levels of triacylglycerol, cholesterol and β–hydroxybutyrate associated with alloxan-induced diabetes mellitus. The aqueous extract tested positive for flavonoids, alkaloids, saponins and cardiac glycosides.*
Conclusion: *From this study, the tuber has proved not only to be an effective hypoglycaemic agent, but also possesses significant ($p<0.05$) hypolipidaemic and hypocholesterolaemic properties while also ameliorating ketosis.*

Keywords: *Alloxan, â-hydroxybutyrate, diabetes, cholesterol, Dioscorea dumetorum, triacylglycerol*

Correspondence should be addressed: *E-mail:* nim-uad@mail.com

Introduction

Dioscorea dumetorum Pax (family: Dioscoreaceae) is commonly known as bitter yam or cluster yam[1]. It occurs wildly throughout Africa, predominantly in the tropics. Its use in treating schistosomiasis[2], as a topical anaesthetic[3] and arrow poison[4] has been reported.

An alkaloid present in the yam extract, dioscoretine, has been reported to possess hypoglycaemic effect[5]. Other alkaloids reported to be present in the yam tuber include dihydrodioscorine[4, 6], dioscorine[7] and dumetorine[4]. Besides alkaloids, the tuber contains small quantities of a sapogenin, diosgenin[4]. Diosgenin is used as a precursor in the commercial synthesis of sex hormones, birth control pills and corticosteroids[8].

In "folk medicine", extracts of *Dioscorea dumetorum* have been used in the treatment of diabetes mellitus because of its hypoglycaemic effect. Diabetes mellitus is a syndrome characterized by chronic hyperglycaemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiencies in insulin secretion and/or insulin action[9]. Impaired carbohydrate utilization in the diabetic leads to accelerated lipolysis, which results in elevated plasma triacylglycerol levels (hyperlipidaemia)[10,11]. The large amounts of fatty acids available to the liver in diabetic patients lead to excess acetyl CoA, which is converted to form ketone bodies. Individuals suffering from untreated diabetes mellitus produce large amounts of ketone bodies in the blood (hyperketonaemia)[10, 11]. The increased availability of acetyl CoA from the β-oxidation of fatty acids are responsible for the hypercholesterolaemia[10, 11].

While the hypoglycaemic properties of this plant are well reported[5, 12], there are however no reports on the effect of the tuber on hyperlipidaemia, hypercholesterolaemia and hyperketonaemia associated with diabetes mellitus despite the relevance of such studies[13]. To reduce the risk of vascular complications in diabetes mellitus, control not only of blood glucose levels but also, lipid levels, blood pressure and weight are necessary[14]. This work was therefore aimed at investigating the effect of the aqueous extract of the *Dioscorea dumetorum* tuber on blood triacylglycerol, cholesterol and β-hydroxybutyrate levels in alloxan-induced diabetic rats.

Experimental

Plant Material

Dioscorea dumetorum tubers collected from a farm at Agenebode, Edo State, Nigeria were peeled, thinly sliced, sun-dried for 6 hr and then oven-dried at 40 ^{o}C till a constant dry weight was recorded. The powdered tuber (50 g) was boiled in 1 litre of distilled water for 10 min and the suspension was filtered. The filtrate was then evaporated to dryness on a rotary evaporator (Gallenkamp, Germany) at 40 ^{o}C. The resulting gummy light brown residue, which weighed 4.03 g (8.1% w/w of dry starting material), was stored overnight at –4 ^{o}C. A 2.5 g portion was reconstituted in 50 ml distilled water (40 ^{o}C) and used as stock crude drug at room temperature (25 ± 2 ^{o}C).

Animals

Twenty-four albino rats of the Wistar strain weighing between (180-250 g) were obtained from the Animal House Unit, Department of Pharmacology, University of Benin, Benin City, Nigeria. The animals were divided into three experimental groups of 8 rats per group, housed in standard rat cages and left to acclimatize to laboratory conditions for two weeks before the commencement of the experiment. Groups 1 and 2 rats were diabetic control and test rats, respectively, while Group 3 rats were normal control. All the rats were maintained on rat pellet food (Pfizer Feeds Plc., Nigeria) and tap water *ad libitum*.

Induction of diabetes in rats

Diabetes was induced within 72 hr in groups 1 and 2 rats by intraperitoneal administration of alloxan monohydrate (Sigma Chemical Company, St. Louis, MO, USA) dissolved in distilled water (5%) in a dose of 100 mg/kg body weight. The blood glucose levels of the hyperglycaemic rats were allowed to stabilize for 3 days[15]. The rats in group 3 (normal control) received distilled water. Diabetes mellitus was confirmed in induced rats by testing for glucosuria using glucose indicator sticks (Bayer Diagnostics, Basingstoke, UK). A 24 hr urine sample collected from each rat was also analyzed for the quantitative estimation of glucose. Glucose levels were determined by the glucose oxidase-peroxidase enzymatic method using the Sigma Diagnostic kit Procedure 510 (Sigma Chemical Company, St. Louis, MO, USA) based essentially on the method of Raabo and Terkildsen[16]. The absorbance values were read in a UV Spectrophotometer (Pye Unicam SP 1800 model). Only rats with fasting blood glucose > 10 mM were considered diabetic and used for further experimentation[17].

Treatment of rats with yam tuber extract

Treatment of the rats with the yam tuber extract began on day 5, post-alloxan treatment. Group 2 rats received oral doses of 400 mg/kg body weight of the stock crude once a day for seven consecutive days using a gavage. The diabetic control rats (Group 1) were given distilled water (orally) in place of the tuber extract. Blood for analyses were collected from the tail tips of conscious rats every other day after an overnight fast, into ice-cold sodium fluoride treated tubes and centrifuged to obtain the plasma.

Biochemical analyses

Total cholesterol in plasma was determined using the Sigma Diagnostic Kit Procedure No. 352 - a modification of the method of Allain *et al.*[18]. Briefly described, cholesterol esters are hydrolyzed and the resulting cholesterol oxidized. The hydrogen peroxide produced is then coupled with the chromogen, 4 - aminoantipyrine and *p* - hydroxybenzene sulphonate in the presence of peroxidase to yield a quinoneimine dye, which has an absorbance maximum of 500 nm. The intensity of colour produced was measured using a Pye Unicam SP 1800 Ultraviolet spectrophotometer. The procedure described by Sigma (No. 337) was employed for the assay of total plasma triacylglycerol, basically a modification of the method of Mc Gowan *et al.*[19]. Briefly, triacylglycerols are hydrolysed by lipase to glycerol and free fatty acids. The glycerol produced is then measured by coupled enzyme reactions catalyzed by glycerol kinase, glycerol phosphate oxidase and peroxidase. The concentration of β-hydroxybutyrate was estimated based upon the enzymatic method described initially by Williamson *et al.*[20], employing Sigma Kits (Procedure No. 310-UV) purchased from Sigma Diagnostics (St. Louis, MO, USA).

Phytochemical Screening

The aqueous extract of *Dioscorea dumetorum* tuber was tested for the presence of secondary metabolites using the procedures of Harborne [21] and Sofowora [22].

Data analysis

Data from blood analyses were expressed in mM as mean $\pm$ SEM. Comparison of the data from the test and control groups of aniamls was carried out using the Student *T*-test[23] at a confidence interval of 95%. A 2-tailed p value < 0.05 was considered statistically significant.

Ethical issues

Prior to the commencement of this study, approval was granted by the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City for the animals to be used in this study.

Results

Rats injected with alloxan monohydrate had several fold increases in the levels of urine and plasma glucose. These were significantly higher ($p < 0.05$) than values obtained for normal control rats (Table 1). Plasma and urine glucose concentrations in excess of 115 mg/100ml and 0.25 g/24 hr, respectively, confirmed the diabetic state[24,25] of the rats used before treatment with the plant extract. The control animals (normal) did not show any significant change in plasma triacylglycerol, cholesterol and β-hydroxybutyrate levels. However, there were significant increases in plasma triacylglycerol, cholesterol and β-hydroxybutyrate concentrations in the diabetic rats when compared with normal control (Tables 2, 3 and 4).

Following one week oral administration of the extract to the diabetic rats (group 2), the percentage reduction in plasma levels of triacylglycerol, cholesterol and β-hydroxybutyrate were 46.63%, 51.54% and 59.43%, respectively.

The phytochemical screening of the aqueous extract of *Dioscorea dumetorum* revealed the presence of flavonoids, alkaloids, saponins and cardiac glycosides.

Table 1: Urine and plasma glucose levels in normal and alloxan-induced diabetic rats before treatment with yam tuber extract

Parameter	Normal rats	Diabetic rats	P-value
Urine glucose [mM]	0.00 (n = 4)	14.13 ± 0.50 (n = 5)	< 0.05
Plasma glucose [mM]	3.89 ± 0.14 (n = 8)	12.78 ± 0.14 (n = 8)	< 0.05

Values are mean ± SEM for plasma and urine samples obtained on day 5 (post-alloxan). The numbers of animals studied are in parenthesis

Discussion

The results of this study clearly indicate that the water extract of *Dioscorea dumetorum* exhibits not only hypoglycaemic effects but also hypocholesterolaemic and hypotriacylglycerolaemic effects in alloxan – induced diabetic rats. The extract also significantly reduced the plasma levels of β-hydroxybutyrate concentrations.

In alloxan-induced diabetes, the β–cells of the islets of Langerhans, which produce insulin, are destroyed[26]. In severe insulin deficiency, there is accelerated lipolysis[10, 11], resulting in the elevated plasma triacylglycerol levels obtained in untreated diabetic rats (Table 2). The excess acetyl CoA produced is a key substrate in the biosynthesis of cholesterol[10, 11], thus leading to the high plasma cholesterol levels observed in untreated diabetic rats (Table 3). High plasma levels of β-hydroxybutyrate in untreated diabetic rats (Table 4) is due to the fact that, in the absence of carbohydrate metabolism, little of the acetyl CoA is metabolized by the tricarboxylic acid cycle, while the remainder is converted to ketone bodies (ketonaemia), and some excreted in urine (ketonuria)[10, 11]. These observations are consistent with previous findings that spontaneous diabetes in man and the experimentally induced condition in animals are characterized by increases in the concentrations of the above biochemical parameters[27, 28].

The hypolipidaemic and hypocholesterolaemic effects of the extract, observed in the diabetic treated rats (Tables 2 and 3), may be due in part to the presence of anti-nutrients. 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[29]. Saponin is known to possess blood cholesterol-lowering activity[29, 30]. The cholesterol-lowering mechanism proposed for saponin is that, it

Table 2: Effect of *Dioscorea dumetorum* extract on total plasma triacylglycerol level (mM) in alloxan-induced diabetic rats. Values are means ± SEM (n = 8)

Day	Diabetic control rats (group 1)	Diabetic treated rats (group 2)	Normal control rats (group 3)	Significance
Pre-alloxan				
0	0.84 ± 0.01 [a]	0.82 ± 0.02 [b]	0.81 ± 0.02 [c]	[ab]p > 0.05; [ac]p > 0.05
Post-alloxan				
3	1.27 ± 0.02 [d]	1.25 ± 0.01[e]	0.81 ± 0.01[f]	[de]p > 0.05; [df]p < 0.05
5	1.84 ± 0.01[g]	1.78 ± 0.02 [h]	0.82 ± 0.02 [i]	[gh]p > 0.05; [gi]p < 0.05
7	1.77 ± 0.01 [j]	1.29 ± 0.01 [k]	0.84 ± 0.01 [l]	[jk]p < 0.05; [jl]p < 0.05
9	1.80 ± 0.01 [m]	1.08 ± 0.01 [n]	0.82 ± 0.01 [o]	[mn]p < 0.05; [mo]p < 0.05
11	1.82 ± 0.01[p]	0.99 ±0.02*[q]	0.80 ± 0.03 [r]	[pq]p < 0.05; [pr]p < 0.05
13	1.82 ± 0.01 [s]	0.95 ± 0.05* [t]	0.78 ± 0.04 [u]	[st]p < 0.05; [su]p < 0.05

*n = 7; Treatment began on day 5

Table 3: Effect of *Dioscorea dumetorum* extract on total plasma cholesterol level (mM) in alloxan-induced diabetic rats. Values are means ± SEM (n = 8)

Day	Diabetic control rats (group 1)	Diabetic treated rats (group 2)	Normal control rats (group 3)	Significance
Pre-alloxan				
0	1.59 ± 0.03[a]	1.61± 0.05[b]	1.55 ± 0.02[c]	[ab]p > 0.05; [ac]p > 0.05
Post-alloxan				
3	2.69 ± 0.01[d]	2.57 ± 0.03[e]	1.57 ± 0.04[f]	[de]p > 0.05; [df]p < 0.05
5	2.90 ± 0.03[g]	2.93 ± 0.02[h]	1.55 ± 0.05[i]	[gh]p > 0.05; [gi]p < 0.05
7	2.98 ± 0.06[j]	2.41 ± 0.02* [k]	1.57 ± 0.03 [l]	[jk]p < 0.05; [jl]p < 0.05
9	3.12 ± 0.03 [m]	1.78 ± 0.09* [n]	1.60 ± 0.02[o]	[mn]p < 0.05; [mo]p < 0.05
11	3.02 ± 0.03 [p]	1.46 ± 0.05* [q]	1.58 ± 0.03 [r]	[pq]p < 0.05; [pr]p < 0.05
13	3.10 ± 0.05 [s]	1.42 ± 0.04* [t]	1.54 ± 0.06 [u]	[st]p < 0.05; [su]p < 0.05

*n = 7; Treatment began on day 5

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[30]. Increased bile acid excretion is offset by enhanced synthesis from cholesterol in the liver and consequent lowering of the plasma cholesterol[31].

In the present study, phytochemical screening of the aqueous extract of *Dioscorea dumetorum* showed the presence of saponins, flavonoids and cardiac glycosides in addition to alkaloids which has previously been extracted and characterized in the yam tuber[4,5,7]. Steroids[32] and sapogenin[4] have also been identified in the yam tuber extract but the presence of these

Table 4: Effect of Dioscorea dumetorum extract on β-hydroxybutyrate level (mM) in alloxan-induced diabetic rats. Values are means ± SEM (n = 8)

Day	Diabetic control rats (group 1)	Diabetic treated rats (group 2)	Normal control rats (group 3)	Significance
Pre-alloxan				
0	0.59 ± 0.10 [a]	0.66 ± 0.11[b]	0.61 ± 0.12[c]	[ab]p > 0.05; [ac]p > 0.05
Post-alloxan				
3	1.86 ± 0.19[d]	1.73 ±0.15[e]	0.68 ± 0.13[f]	[de]p > 0.05; [df]p < 0.05
5	2.52 ± 0.16[g]	2.44 ± 0.18 [h]	0.60 ± 0.11[i]	[gh]p > 0.05; [gi]p < 0.05
7	2.55 ± 0.13 [j]	2.36 ± 0.16* [k]	0.58 ± 0.12[l]	[jk]p > 0.05; [jl]p < 0.05
9	2.99 ± 0.10 [m]	1.74 ± 0.15* [n]	0.60 ± 0.14[o]	[mn]p < 0.05; [mo]p < 0.05
11	2.82 ± 0.10 [p]	1.27 ± 0.2*1[q]	0.66 ± 0.11[r]	[pq]p < 0.05; [pr]p <0.05
13	3.07 ± 0.11[s]	0.99 ± 0.16* [t]	0.68 ± 0.10 [u]	[st]p < 0.05; [su]p < 0.05

*n = 7; Treatment began on day 5

chemical agents was not investigated in this study.

Conclusion

Elevated blood cholesterol, triacylglycerol, β-hydroxybutyrate which occur in diabetic conditions can significantly be reduced by the oral administration of the aqueous extract of *Dioscorea dumetorum* tuber. This finding provides some biochemical basis for the use of the tuber in the management of patients with diabetes and confirm its role as a traditional antidiabetic remedy.

Acknowledgements

The author wishes to express her profound gratitude to Dr. Eric Omogbai of the Department of Pharmacology for his technical assistance and Professors A.U. Osagie and E.A.C. Nwanze for their fatherly encouragement.

References

1. Eka OU. Roots and Tubers. In: Osagie AU, Eka OU (eds). Nutritional Quality of Plant Foods, Benin City, Nigeria, Post-Harvest Research Unit, Department of Biochemistry, University of Benin, 1998, p 4.
2. Steyn DG. An investigation into cases of suspected poisoning in Africans in Northern Rhodesia S Afr Med J 1965; 39: 344.
3. Verdcourt B, Trump E. Common poisonous plants of East Africa. London, England, Collins Publishers, 1969, pp 14-16.
4. Corley DG, Tempesta MS, Iwu MM. Convulsant alkaloids from *Dioscorea dumetorum*. Tetrahedron Lett 1985; 26: 1615-1618.
5. Iwu MM, Okunji CO, Akah P, Tempesta MS, Corley D. Dioscoretine: The hypoglycaemic principle of *Dioscorea dumetorum*. Planta Med, 1990; 56: 119-120.
6. Purseglove JW. Tropical Crops – Monocotyledons. London, England, Longman Publishers, 1976, p 106.
7. Bevan CWL, Broadbent JL, Hirst J. A convulsant alkaloid of *Dioscorea dumetorum*. Nature (London) 1956; 177: 935.
8. Crabbé P. Some aspects of steroid research based on natural products from plant origin. Bulletin of the Society of Chemistry, Belgium 1979; 88: 7
9. Bennett, PH. Definition, Diagnosis and Classification of Diabetes Mellitus and Impaired Glucose Tolerance. In: Kahn CR, Weir GC (eds). Joslin's Diabetes Mellitus, ed 13, Philadelphia, USA, Lea and Febiger, 1994, p193.
10. Granner DK. Hormones of the Pancreas and Gastrointestinal Tract. In: Murray RK, Granner DK, Mayes PA, Rodwell VW (eds). Harper's Biochemistry, ed 24, Connecticut, USA, Appleton and Lange, 1996, pp 586-587.
11. Horton HR, Moran LA, Ochs RS, Rawn JD, Scrimgeour KG. Principles of Biochemistry, ed

2, NJ, USA, Prentice Hall Inc., 1996, pp 474-475.

12. Undie AS, Akubue PI. Pharmacological evaluation of *Dioscorea dumetorum* tuber used in traditional antidiabetic therapy. J Ethnopharmacol 1986; 15: 133-44.
13. Farnsworth NR. "The present and future of pharmacognosy". Newsletter of the American Society for Pharmacognosy, USA, 1980, pp 4-12.
14. Williams G. Management of non-insulin dependent diabetes mellitus. Lancet 1994; 343: 95-100.
15. Jimenez J, Risco, Ruiz T, Zarzuelo A. Hypoglycaemic Activity of *Salvia lavandulifolia*. Planta Medica 1986; 4: 260-62.
16. Raabo E, Terkildsen, TC. On the enzymatic determination of blood glucose. Scand. J. Clin. Lab. Invest. 1960; 12: 402.
17. Al-Awadi F, Fatania H, Shamte U. The effect of a plant mixture extract on liver gluconeogenesis in streptozotocin–induced diabetic rats. Diabetes Research 1991; 18: 163-8.
18. Allain CA, Poon LS, Chan CSG, Richmond W, Fu PC.Enzymatic determination of total serum cholesterol. Clin. Chem.1974; 20: 470.
19. Mc Gowan MW, Artiss JD, Strandberg DR, Zak B. A peroxidase-coupled method for the colourimetric determination of serum triglycerides. Clin. Chem. 1983; 29: 538.
20. Williamson DH, Mellanby J, Krebs HA. Enzymatic determination of D (-) β-hydroxybutyric acid and acetoacetic acid in blood. Biochem. J. 1962; 82: 90.
21. Harborne JB. Phytochemical Methods: A guide to modern techniques of plant analysis, London, England: Chapman and Hall Ltd., 1973.
22. Sofowora EA. Medicinal Plants and Traditional Medicine in Africa, ed 3, Nigeria: Spectrum Books Ltd., 1993, pp 150-153.
23. Woodson RF. Statistical Methods for the Analysis of Biochemical Data. Probability and Mathematical Statistics, Chichester, England: Wiley, 1987, pp 315-6.
24. Henry RJ, Canon DC, Win-Kelman JW. Clinical Chemistry, Principles and Techniques, ed 2, Maryland, USA, Harper and Row, 1974, pp 1265-326.
25. Ellenberg M, Rifkin H. Diabetes Mellitus – Theory and Practice, New York, USA: Medical Examination Publishing Co. Inc., 1983, pp 409-14.
26. Chakravarthy BK, Gupta S, Gode KD. Functional beta cell regeneration in the islets of pancreas in alloxan – induced diabetic rats by (-) – epicatechin. Life Sciences 1982; 31: 2693 -7.
27. West ES, Todd WR, Mason HS, Van Bruggen JT. Textbook of Biochemistry, ed 4, London, England: The Macmillan Company, 1966, pp1118-42.
28. Smith EL, Hill RL, Lehman, IR, Lefkowitz RJ, Handler P, White A. Principles of Biochemistry, ed 7, New York, USA: McGraw-Hill Book Company, 1983, pp 479-83.
29. Thompson LU. Potential Health Benefits and Problems associated with Antinutrients in Foods. Food Research International 1993; 26: 131-149.
30. Sidhu GS, Oakenful DG. A mechanism for the hypocholesterolaemic activity of saponins. Br J Nutr 1986; 55: 643-649.
31. Oakenful DG, Sidhu GS. Could saponins be a useful treatment for hypercholesterolaemia? Eur J Clin Nutr 1990; 44: 79-88.
32. Osagie AU. Phytosterols in Some Tropical Tubers. J Agr Food Chem 1977; 25: 1222.