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Effect of aqueous extract of *Canavalia ensiformis* seeds on hyperlipidaemia and hyperketonaemia in alloxan-induced diabetic rats

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Abstract

The effect of *Canavalia ensiformis* (DC) seeds on diabetic albino rats was studied. Alloxan-induced diabetes mellitus was accompanied by several fold increases in plasma glucose and urinary excretion of glucose. Total plasma triacylglycerol, cholesterol and β-hydroxybutyrate were also significantly increased ($p<0.05$). One-week oral administration of an aqueous extract of the seeds of *C. ensiformis* significantly reduced ($p<0.05$) hyperlipidaemia and hyperketonaemia in the diabetic rats. These results suggest that *C. ensiformis* seeds possess an antidiabetic principle and can be useful for the treatment of diabetes mellitus.

Keywords: *Canavalia ensiformis*, alloxan, diabetes mellitus, triacylglycerol, cholesterol, β-hydroxybutyrate

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INTRODUCTION

Dietary legumes have been reported to influence glycaemia and lipidaemia in man and experimental animals. (Malinow *et al*., 1980; Bingwen *et al*., 1981; Molgaard *et al*., 1987; Donatucci *et al*., 1987). *Canavalia ensiformis* DC (family: Leguminosae) variously known as Jack bean, cat eye, horse bean, one eye bean and overlook, though a native of Central America and West Indies has been widely cultivated in the humid tropics of Africa and Asia. The seeds have been reported to possesss anti-hypercholesterolaemic (Marfo *et al*., 1990) and hypoglycaemic (Enyikwola *et al*., 1991) activities.

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 (Bennett, 1994). The disease is estimated to affect 4-5% of the population (Evans, 1999) and patients are generally classified as either type 1 (insulin-dependent) or type 2 (non-insulin dependent) diabetics. For both types of diabetes, control of blood glucose levels, lipid levels, blood pressure and weight will reduce the risk of vascular problems and associated diseases (Williams, 1994). Earlier work on the effect of *C. ensiformis* on normal and alloxan-induced diabetic rats showed statistically significant decreases in blood and urinary glucose levels. In addition, there was a lower rate of body weight loss in the treated diabetic animals (Nimenibo- Uadia and Osagie, 1999). This study reports the effect of *C. ensiformis* on blood total triacylglycerol, cholesterol and β-hydroxybutyrate in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Animals

Twenty-four Wistar strain albino rats weighing between 180-250g were housed at 25 $\pm$ 2^0C in separate cages (Griffin and George Modular Cage System, Model YSM 580 cage base and YSM 600-540 cage top). They were divided into three groups of 8 animals each and fed *ad libitum* with standard laboratory pellet diet (Pfizer Feeds Plc., Nigeria) and water.

Plant Material

Fifty grams of the pulverized seeds of *C. ensiformis* was boiled in 1 litre of distilled water. After 10 min, the suspension was filtered and the filtrate evaporated to dryness on a rotary evaporator (Gallenkamp, Germany) at 40 ^{o}C. The resulting material was stored overnight at -4^0C and when needed, a 5% portion was reconstituted in water and used as stock crude drug.

Induction of Diabetes

Diabetes was induced in rats within 72 h by the intraperitoneal administration of alloxan monohydrate (Sigma, St. Louis, MO, USA) dissolved in distilled water (5%) in a dose of 100mg/kg body weight (Bailey, 1947; Nimenibo-Uadia and Osagie, 1999). They were then rested for 3 days to allow glucose levels stabilize. Diabetes was confirmed in induced rats by testing for glucosuria using glucose indicator sticks (Bayer Diagnostics, Basingstoke, UK). Fasting glucose levels in blood and a 24h urine sample collected from each rat were determined by the glucose oxidase-peroxidase enzymatic method (Sigma Diagnostics, St. Louis, MO, USA). Only rats with fasting blood glucose >10mmol/L were considered diabetic and

used for further experimentation (Al-Awadi *et al.,* 1991). The diabetic animals were then divided into diabetic control (Group 2) and test rats (Group 3). Animals in Group 1 were untreated rats and served as normal control receiving distilled water only.

Pharmacological Evaluation.

Treatment of test animals with the stock crude drug began on day 5 (post-alloxan). Using a gavage, group 3 rats were orally administered 400 mg/kg body weight of the extract, once daily for seven consecutive days. The diabetic control rats (group 2) and untreated rats (group 1) received distilled water by the same route. Fasting blood samples for analyses were collected from the tail tips of conscious rats every other day after an overnight fast (16h) into ice-cold sodium fluoride treated plastic tubes and centrifuged (MSE minor bench centrifuge). Urinary and plasma glucose levels were assayed using a glucose oxidase kit (Procedure No. 510), purchased from Sigma Diagnostics, St. Louis, MO, USA. Sigma kits were also employed for the colourimetric estimation of total triacylglycerol, cholesterol and β-hydroxybutyrate concentrations in plasma (Procedure Nos. 337, 352 and 310-UV respectively). The intensity of colour produced was measured using a Pye Unicam SP 1800 Ultraviolet spectrophotometer.

Statistical Evaluation

Data were expressed as mean $\pm$ SEM. Differences between the test and control groups of animals were evaluated using student's t-test (Woodson, 1987). $P<0.05$ was considered as statistically significant.

RESULTS

Diabetes mellitus in induced rats was confirmed by fasting blood glucose levels exceeding 10mmol/L (Al-Awadi *et al.,* 1991). The diabetic rats presented with several fold increases in urine and plasma glucose levels. These values were significantly higher ($p<0.05$) than those obtained for normal untreated rats (Table 1).

Table 1: Urine and plasma glucose concentrations in normal and alloxan-induced diabetic rats before treatment with extract (Day 5)

Group	Urine (mmol/L)	Plasma (mmol/L)
Normal	0.00 (n=5)	4.10±0.23 (n=8)
Diabetic	14.63±0.37* (n=5)	13.57±0.32* (n=8)

** As compared with normal rats, $p<0.05$. Values are mean ± S.E.M. Numbers of animals studied are in parenthesis.*

Administration of distilled water to control animals did not produce any significant change ($p>0.05$) on the urinary and plasma glucose, total plasma triacylglycerol, cholesterol and β-hydroxybutyrate levels of either normal or diabetic rats. However, alloxan-induced diabetes mellitus in the rats resulted (Tables 2, 3 & 4) in a spectrum of severity of hypertriacylglycerolaemia (1.29 $\pm$ 0.02 – 1.85 $\pm$ 0.01 mmol/L), hypercholesterolaemia (2.37 $\pm$ 0.04 – 3.05 $\pm$ 0.04 mmol/L) and hyperketonaemia (1.61 $\pm$ 0.18 – 2.69 $\pm$ 0.15 mmol/L). Hyperglycaemic values ranged from 11.10 $\pm$ 0.18 – 17.16 $\pm$ 0.19 mmol/L (laboratory note).

The responses of the diabetic rats to the administration of *C. ensiformis* extract are shown in Tables 2, 3 & 4. After one week, fasting triacylglycerol values declined from

Table 2: Effect of *C. ensiformis* extract on total plasma triacylglycerol concentration in alloxan-induced diabetic rats. (Means ± S.E.M.; number of animals in parenthesis)

Total Plasma Triacylglycerol Concentration (mmol/L) at various times (days)							
			Treatment				
Conditions	**Pre-treatment**		**began** ↓	**Post-treatment**			
	0	**3**	**5**	**7**	**9**	**11**	**13**
Normal Untreated (Group 1)	0.80** ± 0.02 (8)	0.79** ± 0.02 (8)	0.78** ± 0.01 (8)	0.80** ± 0.01 (8)	0.78** ± 0.03 (8)	0.80** ± 0.01 (8)	0.79** ± 0.03 (8)
Diabetic Control (Group2)	Pre-alloxan 0.79** ± 0.03 (8)	Post-alloxan 1.29*** ± 0.02 (8)	1.85* ± 0.01 (8)	1.84* ± 0.01 (8)	1.70* ± 0.01 (7)	1.78* ± 0.01 (7)	1.80* ± 0.03 (7)
Diabetic Treated (Group3)	0.82** ± 0.03 (8)	1.33*** ± 0.01 (8)	1.83* ± 0.01 (7)	1.58*** ± 0.02 (7)	0.91*** ± 0.01 (7)	0.74*** ± 0.02 (7)	0.76*** ± 0.05 (7)

**As compared with normal rats within each group, (Day 0) p<0.05. **As compared with diabetic rats pre-treatment, (Day 5) p<0.05*

Table 3: Effect of *C. ensiformis* extract on total plasma cholesterol concentration in alloxan-induced diabetic rats. (Means ± S.E.M.; number of animals in parenthesis)

Total Plasma Cholesterol Concentration (mmol/L) at various times (days)							
			Treatment				
Conditions	**Pre-treatment**		**began**	**Post-treatment**			
			↓				
	0	**3**	**5**	**7**	**9**	**11**	**13**
Normal Untreated (Group 1)	1.43** ± 0.07 (8)	1.42** ± 0.02 (8)	1.50** ± 0.05 (8)	1.50** ± 0.07 (8)	1.50** ± 0.06 (8)	1.44** ± 0.02 (8)	1.46** ± 0.03 (8)
	Pre-alloxan	Post-alloxan					
Diabetic Control (Group2)	1.50** ± 0.05 (8)	2.42*** ± 0.03 (8)	2.93* ± 0.03 (8)	3.04* ± 0.02 (8)	2.96* ± 0.03 (8)	3.01* ± 0.02 (8)	3.05* ± 0.04 (7)
Diabetic Treated (Group3)	1.53** ± 0.07 (8)	2.37*** ± 0.04 (8)	2.98* ± 0.05 (7)	2.79*** ± 0.02 (7)	2.39*** ± 0.02 (7)	1.92*** ± 0.01 (7)	1.80*** ± 0.03 (7)

**As compared with normal rats within each group, (Day 0) p<0.05. **As compared with diabetic rats pre-treatment, (Day 5) p<0.05*

Table 4: Effect of *C. ensiformis* extract on β-hydroxybutyrate concentration in alloxan-induced diabetic rats. (Means ± S.E.M.; number of animals in parenthesis)

Plasma β-hydroxybutyrate Concentration (mmol/L) at various times (days)							
			Treatment				
Conditions	**Pre-treatment**		**began**	**Post-treatment**			
			↓				
	0	**3**	**5**	**7**	**9**	**11**	**13**
Normal Untreated (Group 1)	0.59** ± 0.12 (8)	0.60** ± 0.14 (8)	0.68** ± 0.14 (8)	0.65** ± 0.14 (8)	0.69** ± 0.11 (8)	0.70** ± 0.12 (8)	0.69** ± 0.11 (8)
	Pre-alloxan	***Post-alloxan***					
Diabetic Control (Group2)	0.68** ± 0.12 (8)	1.70* ± 0.18 (8)	2.16* ± 0.19 (8)	2.69* ± 0.15 (8)	2.51* ± 0.12 (7)	2.50* ± 0.16 (7)	2.64* ± 0.17 (7)
Diabetic Treated (Group3)	0.69** ± 0.10 (8)	1.61**,* ± 0.18 (8)	2.37* ± 0.17 (7)	1.55**,* ± 0.14 (7)	1.14**,* ± 0.05 (7)	0.67**,* ± 0.07 (7)	0.50**,* ± 0.08 (7)

**As compared with normal rats within each group, (Day 0) p<0.05. **As compared with diabetic rats pre-treatment, (Day 5) p<0.05*

1.83 ± 0.01 to 0.76 ± 0.05 mmol/L (a 58.5% reduction), cholesterol levels were reduced from 2.98 ± 0.05 to 1.80 ± 0.03 mmol/L (a 39.6% reduction) while β-hydroxybutyrate levels fell from 2.37 ± 0.17 to 0.50 ± 0.08 mmol/L (a 78.9% reduction).

DISCUSSION

The statistically significant lowering of plasma concentrations of triacylglycerol, cholesterol and β-hydroxybutyrate in alloxan-induced diabetic rats by oral administration of the aqueous extract of the seeds of *C. ensiformis* indicates that the extract exhibits antihyperlipidaemic and antihyperketonaemic properties.

In alloxan-induced diabetes, the β-cells of the islets of Langerhans, which produce insulin, are destroyed (Chakravarthy *et al*., 1982). Hyperglycaemia occurs because the insulin dependent tissues are unable to take up and utilize plasma glucose and also because of the enhanced hepatic gluconeogenesis from amino acids derived from muscle proteins (Rang *et al*., 1995; Granner, 1996). In severe insulin deficiency, there is accelerated lipolysis, which results in elevated plasma triacylglycerol levels (hyperlipidaemia), as observed in the untreated diabetic rats (Table 2). The hypercholesterolaemia observed in the untreated diabetic rats (Table 3), is due to the high concentration of acetyl-CoA obtained from the increased β-oxidation of fatty acids, acetyl-CoA being a key substrate in the biosynthesis of cholesterol (Granner, 1996). Ketosis occurs in the absence of insulin because there is increased fat breakdown to acetyl-CoA. In the absence of carbohydrate metabolism, acetyl-CoA is converted to aceto-acetate, β-hydroxybutyrate and acetone (Rang *et al*., 1995), hence the elevated plasma concentration of β-hydroxybutyrate seen in the untreated diabetic rats (Table 4). The observations in this study are consistent with previous reports that spontaneous diabetes in man as well as the experimentally induced condition in animals is characterized by elevations in the concentrations of the above biochemical parameters (West *et al.,* 1966; Smith *et al.,* 1983).

Saponin among other secondary metabolites has been found present in the aqueous extract of *C. ensiformis* (Nimenibo-Uadia and Osagie, 1999). This may be responsible for the lowering effect of this extract on blood lipids. Saponin is known to possess blood cholesterol lowering activity. Saponins may lower cholesterol by binding with cholesterol in the intestinal lumen, preventing its absorption, and/or by binding with bile acids, causing a reduction in the enterohepatic circulation of bile acids and an increase in its faecal excretion (Sidhu and Oakenful, 1986). Increased bile acid excretion is offset by enhanced bile acid synthesis from cholesterol in the liver and consequent lowering of the plasma cholesterol (Oakenful and Sidhu, 1990).

This study has shown that, oral administration of the aqueous extract of *C. ensiformis* seeds can significantly reduce not only urinary and blood glucose levels, but also elevated levels of triacylglycerol, cholesterol and ketone bodies associated with diabetes mellitus. These properties justify its use by traditional healers as an antidiabetic agent.

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