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South African Journal of Botany 80 (2012) 9 – 12

SOUTH AFRICAN JOURNAL OF BOTANY

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The hypoglycemic activity of *Euclea undulata* Thunb. var. *myrtina* (Ebenaceae) root bark evaluated in a streptozotocin–nicotinamide induced type 2 diabetes rat model

M.S. Deutschländer [a,*], N. Lall [a], M. Van de Venter [b], S. Dewanjee [c]

[a] *Department of Plant Science, University of Pretoria, Pretoria 0002, South Africa*
[b] *Department of Biochemistry and Microbiology, P.O. Box 77000, Nelson Mandela Metropolitan University, Port Elizabeth 6031, South Africa*
[c] *Advance Pharmacognosy Research Laboratory, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700032, India*

Received 15 December 2011; received in revised form 2 February 2012; accepted 6 February 2012

Abstract

The hypoglycemic activity of a crude acetone extract of the root bark of *Euclea undulata* var. *myrtina* was evaluated in a streptozotocin–nicotinamide induced type 2 diabetes rat model after positive results were obtained by *in vitro* screening of glucose utilization by C2C12 myocytes, 3T3-L1 preadipocytes and Chang liver cells and alpha-glucosidase inhibition. Thirty male Wistar rats were used for the experiment. Type 2 diabetes was induced by a single intraperitoneal injection of streptozotocin and administration of nicotinamide 15 min after. Animals exhibiting fasting glucose levels of 140–200 mg/dl after 7 days were screened as type 2 diabetes. Extract was administered for 21 days orally at a concentration of 50 mg/kg and 100 mg/kg respectively. Glibenclamide (1 mg/kg) was used as positive control. On day 21, blood lipid profiles and body weight were determined by using standard enzymatic colorimetric kits before the rats were sacrificed by cervical decapitation. The crude acetone extract of *E. undulata* root bark at a concentration of 100 mg/kg body weight significantly lowered fasting blood glucose levels as well as elevated cholesterol and triglyceride levels to near normal without any weight gain. The results indicate that the crude acetone root bark extract of *E. undulata* exhibit antidiabetic activity in type 2 induced diabetic rats. It confirms the *in vitro* screening results as well as its use in the treatment of diabetes by traditional healers and herbalists in southern Africa.

Keywords: *Euclea undulata*; Hypoglycemic activity; Streptozotocin–nicotinamide induced diabetic rats

1. Introduction

Diabetes mellitus is an in-curable metabolic disease managed and treated in first world countries by using conventional synthetic drugs. In many third world and developing countries however, diabetic patients make use of traditional medicinal herbs and remedies as it is more easily accessible and affordable (Agarwal, 1985). Diabetes mellitus is characterized by hyperglycemia and glucose intolerance associated with abnormalities in carbohydrate, protein and fat metabolism due either to total or partial insulin deficiency, or to the impaired effectiveness of insulin's action, or to a combination of both (O'Brain and Granner, 1991) The number of individuals with type 2 diabetes in developing countries has increased due to the adoption of a western diet and life style and is a growing concern (Lehohla, 2006). Diabetes is associated with an increase in ischemic heart disease, stroke, hypertensive disease, renal failure, blindness and other debilitating diseases. According to a survey done by Bradshaw et al. (2007), 5.5% of the people in South Africa in the age group 30 years and older are diabetic and the number increases with age. They attributed 14% of ischemic heart disease, 10% of stoke, 12% of hypertensive disease and 12% of renal disease to diabetes. The World Health Organization (WHO) estimated that in 1998 there were 135 million people with diabetes, 171 million in 2000 and it has been projected to increase to 366 million in 2030 (Bradshaw et al., 2007).

* Corresponding author. Tel.: +27 12 4204046; fax: +27 12 4206668.
E-mail address: miranda.deutschlander@up.ac.za (M.S. Deutschländer).

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doi:10.1016/j.sajb.2012.02.006

This increase in the number of individuals with type 2 diabetes has resulted in a renewed interest in the use of natural and traditional remedies for treating diabetes (Vesudevan and Garber, 2005).

Euclea undulata Thunb. var. *myrtina* (Ebenaceae) (common guarri), a dense, erect, dioecious shrub or small tree is being used by traditional healers and herbalists in the Venda area in the treatment of diabetes. An aqueous infusion is being made with ground root bark and drank as a tea. It was selected for the identification of bio-active principles after preliminary *in vitro* screenings were done for hypoglycemic activity on an acetone extract of the root bark. This selection was based on the facts that the crude acetone extract of *E. undulata* root bark gave positive results (hypoglycemic activity) in the *in vitro* assays done on C2C12 myocytes, 3T3-L1 preadipocytes and in Chang liver cells without displaying any toxicity and scored a +3 according to the scoring system developed by Van de Venter et al. (2008). The carbohydrate-hydrolyzing enzymes alpha-amylase and alpha-glucosidase were also inhibited to some extent (Deutschländer et al., 2009). The exact mechanism of action still needs to be investigated. It is possible that it might be two fold due to the presence of two isolated compounds from the crude acetone extract namely the flavonoid epicatechin that showed the potential to lower blood glucose levels in an *in vitro* assay on C2C12 myocytes and the triterpene α-amyrin-3O-ß-(5-hydroxy) ferulic acid that had the ability to inhibit alpha-glucosidase (Deutschländer et al., 2011).

2. Materials and methods

2.1. Plant material

Plant material was collected at De Wagensdrift, Gauteng, South Africa in August 2005. GPS coordinates S 25°22′11,15″ and E 28°22′52,0″. Voucher specimens (Deutschländer nr 95254) have been deposited at the H.G.W.J. Schweickert Herbarium, University of Pretoria and authenticated by Ms M. Nel.

2.1.1. Extraction of the plant material

Plant material was air dried and the root bark stripped from the roots before it was ground. The ground root bark (215 g) was soaked in 0.5 l acetone for three days while on a shaker. After three days the extract was filtered and the residue was extracted again with fresh acetone (3×). The plant extracts were combined and evaporated using a rotary evaporator to yield 87 g (40%) total extract.

2.2. Animals

Thirty healthy male Wistar rats between 2 and 3 months old, weighing 180–200 g were used for the execution of this study. Animals were housed in standard polypropylene cages (4 per cage) and maintained under standard laboratory conditions (12 h light-dark cycle; temperature 20±2 °C; relative humidity 50±15%). They were fed a standard rat pellet diet (Hindustan Liver Ltd. Mumbai, India) and had access to water *ad libitum*. The principles of Laboratory Animal care (Public Health Services, 1986) were followed throughout the duration of the experiment.

2.3. Chemicals

Streptozotocin used for the induction of diabetes in the Wistar rats was obtained from SISCO Research Laboratory PVT. Ltd. India and nicotinamide was purchased from Qualigens Fine Chemicals, Division of Glaxo, Mumbai, India. The other reagents used for the execution of the experiment were of analytical grade. Glibenclamide (Daonil™, Hoechst, India) used as positive control, was purchased from a local medical store, Jadavpur, India.

2.4. Rat antihyperglycemic assays

2.4.1. Induction of diabetes

Hyperglycemia was induced in overnight fasted adult male Wistar rats weighing between 180 and 200 g by a single intraperitoneal injection of 65 mg/kg streptozotocin in a citrate buffer (pH 4.5) to a volume of 1 mg/kg body weight (Siddque et al., 1987; Maiti et al., 2009) and the administration of nicotinamide (110 mg/kg i.p) 15 min later (Masiello et al., 1998). Animals exhibiting fasting glucose levels of 140–200 mg/dl after 7 days were screened as type 2 diabetic rats and used for the experiment (Dewanjee et al., 2009).

2.4.2. Experimental design

Animals were divided into five groups of six rats each. The vehicle and extract were administered orally for 21 days.

Group 1: Normal control rats

Table 1
Effect on fasting blood glucose level in streptozotocin–nicotinamide induced diabetic rats.

Group	Fasting blood glucose level (mg/dl)					
	Initial	1st day	4th day	7th day	14th day	21st day
Normal control	71.00±1.32	71.67±1.54	70.83±1.54	71.83±1.20	71.67±1.28	71.50±1.50
Diabetic control	160.83±6.51 *	162.5±6.45 *	167.83±6.70 *	169.00±5.13 *	171.33±5.13 *	171.83±4.28 *
Diabetic+test drug (50 mg/kg)	160.17±5.81	152.83±5.88	144.67±5.39 **	140.33±6.72 ***	133.33±5.74 ***	128.00±5.73 ***
Diabetic+test drug (100 mg/kg)	164.83±5.61	147.83±7.29	128.67±7.83 ***	124.67±6.83 ***	117.67±5.96 ***	114.33±6.32 ***
Diabetic+glibenclamide (1 mg/kg)	161.67±4.27	141.33±4.78	119.33±4.06 ***	116.33±3.91 ***	108.67±4.28 ***	107.50±5.10 ***

Values are expressed as mean±S.E.M. (n=6).
* $p<0.01$ when compared with normal control.
** $p<0.05$ when compared with diabetic control.
*** $p<0.01$ when compared with diabetic control.