

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

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Abstract

The hypoglycaemic 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 minutes 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 (1mg/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 100mg/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.

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 hyperglycaemia 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'Brien 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 ischaemic 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 ischaemic heart disease, 10% of stroke, 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). 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).

E. 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 drunk as a tea. It was selected for the identification of bio-active principles after preliminary *in vitro* screenings were done for hypoglycaemic 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 (hypoglycaemic 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-hydrolysing enzymes alpha-amylase and alpha-glucosidase were also inhibited to some extent (Deuschländer *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 flavanoid epicatechin that showed the potential to lower blood glucose levels in an *in vitro* assay on C2C12 myocytes and the triterpene α -amyrin-3O- β -(5-hydroxy) ferulic acid that had the ability to inhibit alpha-glucosidase (Deuschländer *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 (Deuschländer 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 extracted again with fresh acetone (3X). The plant extracts were combined and evaporated using a rotatory evaporator to yield 87 g (40 %) total extract.

2.2 Animals

Thirty healthy male Wistar rats between 2-3 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 $50 \pm 15\%$). 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 (DaonilTM, Hoechst, India) used as positive control, was purchased from a local medical store, Jadavpur, India.

2.4 Rat antihyperglycaemic assays

2.4.1 Induction of diabetes

Hyperglycaemia was induced in overnight fasted adult male Wistar rats weighing between 180-200 g by a single intraperitoneal injection of 65 mg/kg streptozotocin in a citratebuffer (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 minutes 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 was administered orally for 21 days.

Group 1: Normal control rats

Group 2: Diabetic control rats

Group 3: Diabetic rats administered test drug at a dose of 50 mg/kg

Group 4: Diabetic rats administered test drug at a dose of 100 mg/kg

Group 5: Diabetic rats administered glibenclamide at a dose of 1 mg/kg

Blood samples were drawn by retro-orbital puncture and fasting glucose levels were estimated on days 0, 1, 4, 7, 14 and 21 by using a single touch glucometer (Ascensia Entrust, Bayer Health Care, USA). Blood lipid profiles and body weight were determined on day 21 by using standard enzymatic colorimetric kits (Span

Diagnosics Ltd., Surat, India) after which the rats were sacrificed by intervention by cervical decapitation.

2.5 Statistical analysis

Data were statistically evaluated by using one way ANOVA, expressed as mean \pm S.E.M. The results were further analyzed by applying Dun-nett's t-test using GraphPad InStat version 3.05, Graph pad software, U.S.A. A p-value < 0.05 was considered to be significant (Maiti *et al.*, 2009).

3. Results and discussion

The crude acetone extract of *E. undulata* var. *myrtina* at a concentration 100 mg/kg body weight significantly lowered fasting blood glucose levels of type 2 diabetic rats when compared with the diabetic control rats (Table 1). Treatment with the crude acetone extract at a concentration of 100 mg/kg body weight of *E. undulata* significantly lowered the elevated cholesterol and triglyceride levels to near normal status when compared with the diabetic control group (Table 2). The effect of the crude acetone extract on the final body weight of streptozotocin-nicotinamide induced diabetic rats is given in Table 3. The results indicated that there were no significant weight gains or losses after 21 days in the diabetic rats treated with the *E. undulata* crude acetone extract at a concentration of 100 mg/kg body weight. The results indicate that the crude acetone rootbark extract of *E. undulata* exhibit antidiabetic acitivity in type 2 induced diabetic rats and confirms the *in vitro* assay results on C2C12 myocytes, 3T3-L1 preadipocytes and Chang liver cells. The crude acetone extract (at 50 μ g/ml) lowered blood glucose levels in C2C12 myocytes 62.2%, 3T3-L1 preadipocytes 26.0% and in Chang liver cells 19.7% (Deuschländer *et al.* 2009). Phytochemical examination coupled with bioassay-guided fractionation of the crude acetone extract led to the isolation of four compounds of which epicatechin has the ability to lower blood glucose levels 82.8% in C2C12 myocytes and α -amyrin-3O- β -(5-hydroxy) ferulic acid has the ability to inhibit alpha-glucosidase 68% (Deuschländer *et al.* 2011).

Insert tables 1, 2 and 3

4. Conclusions

Phytochemical studies conducted on *Euclea* species by Costa *et al.* (1978) demonstrated the presence of triterpenoids in the stems and leaves. Two naphthoquinones, diospyrin and 7 methyl-juglone, were isolated from the root, stem and fruit of *E. undulata* var. *myrtina* by Van der Vyver and Gerritsma (1973; 1974). Chemical analysis indicated the presence of 3.26 % tannins in bark, saponins and reducing sugars in leaf and stems, but no alkaloids, naphthoquinones or cardiac glycosides (South African National Biodiversity Institute, 2005). Deuschländer *et al.* (2011) isolated a new α -amyrine-3O- β -_5-hydroxy) ferulic acid, the triterpenes betulin and lupeol and the flavanoid epicatechin from the crude acetone root bark extract from *E. undulata*.

The *in vivo* results obtained in streptozotocin-nicotinamide induced type 2 diabetic rats indicated that the crude acetone extract of *E. undulata* at a concentration of 100 mg/kg body weight has the ability to lower blood glucose levels as well as normalize elevated cholesterol and triglyceride levels when compared to that of the diabetic control group. The *in vivo* results obtained with the crude acetone extract of *E. undulata* corroborate the *in vitro* results that the crude acetone root bark extract of *E. undulata* has the ability to lower blood glucose levels (Deuschländer *et al.* 2009). These findings confirm the ethnomedicinal use of *E. undulata* by traditional healers for the treatment of diabetes. Histological analysis should however still be done on the vital organs such as the pancreas and liver to determine if the crude acetone extract are responsible for any histopathological changes in these organs.

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

Group	Fasting blood glucose level (mg/ dl)					
	Initial	1 st day	4 ^h day	7 th day	14 th day	21 th 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 ^b	140.33 ± 6.72 ^a	133.33 ± 5.74 ^a	128.00 ± 5.73 ^a
Diabetic + Test drug (100 mg/kg)	164.83 ± 5.61	147.83 ± 7.29	128.67 ± 7.83 ^a	124.67 ± 6.83 ^a	117.67 ± 5.96 ^a	114.33 ± 6.32 ^a
Diabetic + Glibenclamide (1 mg/kg)	161.67 ± 4.27	141.33 ± 4.78	119.33 ± 4.06 ^a	116.33 ± 3.91 ^a	108.67 ± 4.28 ^a	107.5 ± 5.10 ^a

Values are expressed as mean ± S.E.M. (n=6).

* $p < 0.01$ when compared with normal control.

^b $p < 0.05$ when compared with diabetic control.

^a $p < 0.01$ when compared with diabetic control.

Table 2. Effect on blood lipid profile (after sacrifice on day 21) in streptozotocin-nicotinamide induced diabetic rats.

Group	Blood lipid level (mg/ dl)	
	Cholesterol	Triglycerides
Normal Control	54.24 ± 1.88	52.17 ± 1.02
Diabetic Control	92.13 ± 3.21*	102.76 ± 2.12*
Diabetic + Test drug (50 mg/kg)	78.67 ± 2.04 ^a	87.67 ± 3.13 ^a
Diabetic + Test drug (100 mg/kg)	71.33 ± 1.22 ^a	76.50 ± 2.54 ^a
Diabetic + Glibenclamide (1 mg/kg)	57.50 ± 2.39 ^a	68.65 ± 1.92 ^a

Values are expressed as mean ± S.E.M. (n=6).

* $p < 0.01$ when compared with normal control.

^b $p < 0.05$ when compared with diabetic control.

^a $p < 0.01$ when compared with diabetic control.

Table 3. Effect on initial and final body weight of streptozotocin-nicotinamide induced diabetic rats.

Group	Weight (gm)	
	Initial	21 st day
Normal Control	165.00 ± 4.08	169.17 ± 4.36
Diabetic Control	152.50 ± 6.55	136.50 ± 5.18
Diabetic + Test drug (50 mg/kg)	159.17 ± 3.75	149.17 ± 5.83
Diabetic + Test drug (100 mg/kg)	157.50 ± 8.92	152.67 ± 7.46
Diabetic + Glibenclamide (1 mg/kg)	157.00 ± 4.95	155.83 ± 4.55

Values are expressed as mean ± S.E.M. (n=6).