Advances in Science and Technology, Vol. 4, No. 2, September / October 2010, pp 120 - 124

Anti-Diabetic Activity of *Tetracarpidium Conophorum* Muell Arg. (Hutch & Dalz) Ethanolic Seed Extract on Diabetic Rats

C.S. Iwuoha Odoemena[1]*, I.R. Udosen[2] and S.M. Sam[3]

[1]Department of Botany and Ecological Studies, University of Uyo, P.M.B. 107, Uyo, Nigeria.
[2]Department of Biology, College of Education, Afaha Nsit, Akwa Ibom State, Nigeria. [3]Department of Plant Science and Biotechnology, University of Port-Harcourt P. M. B. 5323 Choba, Port Harcourt, Nigeria.

(Submitted: May 24, 2010; Accepted: October 6, 2010)

Abstract

The anti-diabetic activity of ethanolic seed extract of *Tetracarpidium conophorum* (African walnut) was studied on alloxan-induced diabetic rats. The LD_{50} of the extract was determined to be 287.2 mg/kg and a single dose of 250 mg/kg body weight of the extract was intraperitoneally administered as the treatment dose and the blood glucose levels examined for 12 h at 2 h intervals. The extract exhibited significant ($P < 0.05$) reduction in the blood glucose levels of the rats. The extract compared favourably with the standard reference drug (glibenclamide) which all gave their maximum blood glucose level reduction at 6 h duration. The confirmation of anti-diabetic potentials of the Tetrac8fpidium conophorum seed has been justified in this study.

Keywords: *Tetracarpidium conophorum* seed, hypoglycaemic activity, diabetic rats

1.0 Introduction

Diabetes mellitus is a major disease characterized by derangement in carbohydrate, fat and protein metabolism resulting in hyperglycaemia. This disorder is as a result of insufficient production of insulin or inefficient insulin utilization (Aka *et al.*, 2002).

In most developing countries including Nigeria, most diabetic patients are finding it increasingly' difficult to manage hyperglycaemic condition due to high cost of synthetic anti diabetic drugs like sulfonylurea, biguanides and intravenous insulin injections which also have their side effects (Berger, 1985). Another problem associated with the orthodox drugs is the vitiation of therapeutic efficacy of some of the drugs in the maintenance of normoglycaemic levels. This therefore calls for more biomedical research on hypoglycaemic substitutes for the control of the disease.

Some of the plants used by the population as anti-diabetic remedies are edible plants which have added further interest in their study because of their dual control factors as food and medicine for the management of diabetes. A menu including these types of plants could be developed for diabetic patients in order to improve their diet and control the disease.

Some Nigeria medicinal plants have been associated with the management and control of diabetes, but still, diabetes and its related complications continue to be a major medical problem in Nigeria (Nimenibo-Uadia, 2003; Manago and Ugbomeh, 2004; Okeke and Elekwa, 2006; Odoemena *et al.*, 2007).

Tetracarpidium conophorum (African walnut) of the family. Euphorbiaceae is a forest scramble with about six seeds contained in a compartmentalized pod. The seed is one of the walnut foods consumed in south eastern Nigeria, It is commonly known "ukpa" by the Ibos and "asala" by the Yorubas. The seed has a delicious taste which becomes bitter to the mouth if water is drunk shortly after consumption. In southern Nigeria ethnomedicine, the seed is used to engance male fertility and as an anti-diabetic agent while the leaves are used for the treatment of dysentery. Also the seed is believed to reduce the risk of developing breast cancer in women. It is also claimed to improve endothelial' functions in hypercholesteromic subjects. The plant is an ·anti-biotic agent (Ajaiyeoba and Fadara 2006). The need

Corresponding Author's E-mail: sonnyodo60@yahoo.co.uk

for the development of newer hyperglycaemic agents is imperative and therefore, the purpose of this study was to carry out a scientific verification of the anti diabetic potential claims on *Tetracarpidium conophorum* seed by the consumers.

2.0 Materials and Methods

The *Tetracarpidium conophorum* seeds used for this study were extracted from the air-dried pods of the plant, purchased from Abayi Umuocham local market in Aba North Local Government Area of Abia State, Nigeria. The seeds were thoroughly washed and dried under room temperature before milling to a coarse powder. 200 g of the milled seed was soaked in 500 ml of 70% ethanol for 72 h with intermittent shaking. The resulting mixture was filtered using glass wool through a separatory funnel. The filtrate obtained was concentrated *in vacuo* at 40°C using a rotary evaporator giving a yield of 17 .8 g of the solid extract.

2.1 Phytochemical Screening of the Seed Extract

A preliminary phytochemical screening of the seed extract was carried out according to the methods of Trease and Evans (1989) and Sofowora (1993), to test for the presence or absence of medicinal bioactive chemical constituents.

2.2 Animals

Thirty five animals used in the study were adult male and female wister albino mice (200 – 300 g) obtained from the University of Uyo animal house, Uyo, Nigeria. The animals were used after an acclimatization period of. 7 days to room temperature of 27+ 5°C and 'relative humidity of 50%. They were housed in standard cages and maintained on standard animal pellets and water *ad libitum*.

2.3 Acute Toxicity Test

The LD_{50} of the extract was determined in mice intraperitoneally (IP) using the method of Dietrich (1983).

2.4 Determination of Blood Sugar Levels in Rats

Two groups of 5 rats each labeled group I and II were used to test the normoglycaemic effect of the extract. Group I rats served as the control and received equi-volume (per kg body weight) of normal saline (2 ml/kg) and group II received a single dose of 250 mg/kg of the seed extract dissolved in a normal saline, all administered intraperitoneally (IP).

The rats were fasted 'for 12 hours after administration of the extract and thereafter the blood glucose level of each rat in the two groups were measured after 2 hour intervals for 12 hours. The blood was obtained by giving a light cut at the tip of the rats tail and fresh blood squeezed out to the sensor pad of a specified strip of the glucose measuring meter (Glucometer Gx Model, USA).

The hypoglycaemic effect of the extract was also tested on another set of' 20 rats labelled groups III, IV, V and VI. The animals were made hyperglycemic (diabetic) by intraperitoneal administration of a single dose of 15 mg/kg each of alloxan monohydrate, freshly prepared as a 10% solution· in saline water. The rats were freely fed for 7 days and on the 8th day the survivors were fasted for 12 hours. Three days after, the diabetic induced rats in group IV were treated with a single dose of 250 mg/kg of the ethanol crude extract of *Tetracarpidium conophorurn* through IP route while group III animals were treated with equi-volume per kg body weight of normal saline to serve as the untreated hyperglycaemic control group. Similarly, group VI animals were given 10 mg/kg of standard diabetic drug (glibenclamide) to serve as reference standard treatment. . The blood sugar levels were measured at 2 hour intervals for 12 hours according to the procedure of Odoemena *et al. (*2007).

2.5 Statistical Analysis

The mean blood sugar levels of the rats were expressed as mg/dl ±SEM, and, the student's t-test was used' to test significance of difference between treated groups and the controls with P = 0.05 (Steel and Torrie 1980).

3.0 Results

The fresh ethanolic seed extract of *Tetracarpidium conophorum* gave positive chemical reactions for alkaloids, saponlns, tannins, flavonoids, anthraquinone and proteins.

The maximum reduction in blood sugar level

occurred at 250 mg/kg of the extract dose which significantly ($p < 0.05$) reduced the blood sugar level in fasted normal rats from a mean value of 87.28±134 mg/dl at O h to 58.01±2.56mg/dl at 6 h (Figure 1).Thereafter the blood sugar gradually increased to 63.20 2.06mg/dl within the 12 h duration . Normal saline caused no significant ($p < 0.05$) reduction in the blood sugar level.

The effects of the extract, glibenclamide and normal saline on the blood sugar levels of alloxan induced diabetic rats are shown in Table 1. The extract compared favourably with the reference diabetic drug (glibenclamide 10 mg/kg) in that the least blood sugar levels were obtained at 6 h intervals with values of 82.51 ±2.81 and 79.23 ± 1.67 mg/dl respectively. Both values were not significantly ($p < 0.05$) different from each other, but were significantly higher than those of the normal saline control treatment:

The bioassay investigation on the ethanolic seed extract of *Tetracarpidium conophorum* showed that the seed extract caused significant ($p < 0.05$) reduction in the blood sugar levels in the experimental rats. In both the normoglycaemic and alloxan diabetic rats, the extract was found to produce a marked reduction in blood sugar, which became significant

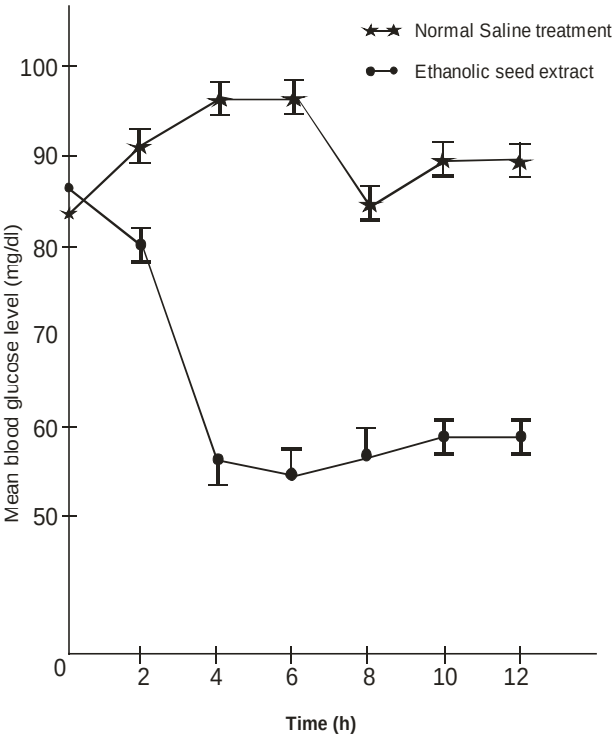

Figure 1: Effect of *Tetracarpidium conophorum* ethanol seed extract (250mg/kg IP) on normaglycaemic rats.

after 2 hours and hard persisted through to 6 h. In all the experimental groups, prior to the alloxan administration, the basal levels of blood glucose of the rats were not significantly different. However, 2

Table 1: Effect of *Tetracarpidium conophorum* ethanolic seed extract (250 mg/kg) on blood glucose level in alloxan induced diabetic rats.

		Time						
Animal groups	Treatment	Oh	2h	4h	6h	8h	10h	12h
III	Normal rats treated with saline water as control	87.6±3.36	90.72.±2.48	83.13±2.48	90.24±1.82	82.56±1.36	79.23±2.41	92.86±1.52
IV	Induced diabetic rats treated with saline water	86.9±2.11	135.8±4.21	145.61±2.54	180.6±2.67	168.3±3.37	152.4±2.82	171.5+3.50
V	Diabetic rats Treated with 250mg/kg of Extract	89.4 2.71	120.5 3.66	102.1 2.55	82.5 2.81	84.6 1.62	90.35 2.26	92.18 2.80
VI	Diabetic rats Treated with 10mg/kg of Glibenclamide	78.7 ±3.20	110.51 ±2.80	98.3 ±1.54	79.2 ±1.67	82.7 ±3.21	89.56 ±1.30	102.36 ± 2.12

Values represent mean ± SEM of 5 replicate determinations; p = 0.05

hours after alloxan treatment, the blood glucose levels were significantly higher in the rats selected for the study (Table 1).

Despite the reduction in the blood sugar level of the rats by the extract, the extract also retained its potent and progressive activity when compared with that of the normal saline control and pretreatment values.

4.0 Discussion

The use of traditional medicine and medicinal plants in Nigeria as a normative basis for the maintenance of good health has been widely observed (Uhegbu and Ogbuehi, 2004; Gyang *et al*, 2004; Odoemena *et al.*, 2008).

The preliminary identification of the previously mentioned alkaloids and flavonoids and their co-existence with the phenolic acids may explain at least in part some of the anti-diabetic and anti-oxidative properties of the extract. These findings are in agreement with some earlier reports by *Fushiya et al. (1999), Gyang et al.* (2004) and Odoemena *.et al.* (2007) who were who were able to isolate and study the suppressive effect of flavonoid and glycoside fractions of different plant extracts on diabetic rats. The non significant reduction in the blood glucose level by glibenclamide and the seed extract is a clear indication that the seed is an anti-diabetic regimen. In glucose-loaded animals, the extract was able to reduce the blood glucose to the normal level and it is possible that the drug might be acting by potentiating the pancreatic secretion or increasing the glucose uptake.

5.0 Conclusion

In conclusion, the seed extract of *T. Conophorum* showed significant anti-diabetic effect in diabetic rats after IP administration, thus lending credence to claims by traditional healers and consumers of walnut regarding its efficacy in checking diabetes in man.

References

Ajaiyeoba, E.O. and Fadara, D.A. 2006, "Antimicrobial potential of extracts and fractions of the African walnut *Tetracarpidium conophorum*", African Journal of Biotechnology **5** (22), 2322 - 2325.

Akah, P. A, .Okoli, C.O. and Nwafor, S.V. 2002, "Phytotherapy in the management of diabetes mellitus", J. *Natural Remedy* **2,** 1-10.

Berger, W.C. 1985, "Incidence of severe side effects during therapy with sulfolnylureas and biguanide", Hormone metab. Res. Suppl. **15,** 111-115.

Dietrich, L. 1983, "A new approach to practical acute toxicity testing", Arch. Toxicol. **54,** 275-287.

Fushiya, S., Kishi, Y., Hattori K., Batkhuu, J., Takano, F., Singab. A.N., Okuyama, T. 1999 "Flavonoids from *Cleome droserifolia* suppress No. production in activated macrophages in vitro", Planta Med. **65,** 404 - 407 .

Gyang, S.S., Nyam, D.D. and Sokomba, E.N. 2004, "Hypoglycaemic activity of *Vernonia amygdelina* (Chloroform extract) in normoglycaermic and alloxan induced hyperglycaemic rats", J. Pharmacy and Bioresources **1**(1), 61 - 66.

Monago, C. C. and Ugbomeh, P.A. 2003, "Anti-diabetic effect uf Emilia *sanchifora* in dithazone Diabetic rats", Global Journal of Pure and Applied Sciences **10,** 183-187.

Nimenibo- Uadia, R. 2003, "Flcus exasperate: Effects on Diabetes mellitus in an experimental rat model", Global Journal of Pure and Applied Sciences **9**(4), 592-532.

Odoemena C.S.I., Ekpo, B.A.J. and Luke, M.I. 2007, "Hypoglycaemic activity of *Ocimum gratissimum* (Linn) etllanolic leaf extract in normoglycaemic and hyperglycaemic rats", J. Tropical Bioscicnces **7,** 24-28.

Odoemena, C.S.I., Ekanem, B.E . and Uboh, O.G. 2008, "Effect of *Ageratum conyzoides* L. Leaf extract on blood coagulation and platelet count in Albino rats", Nigerian Journal of Botany **21**(1), 187-193.

Okeke, C.U. and Elekwa, I. 2006, "Comparative hypoglycemic effects of three Nigerian vegetable spices, *Gongronema Latifolium Benth, Allium satvrum Linn, and Ocimum gratissimum* Linn, on alloxan induced diabetic rats", Global Journal of Pure and Applied Sciences **19**(1), 138 – 146.

Sofowara, A. 1993, "Medicinal plants and Traditional Medicine in Africa", (2nd Ed). Spectrrum Books Ltd. Nigeria

Trease, G.E. and Evans, W.C. 1989, "Pharmacognosy", (13th Ed). Bailliere Tindall

London.

Steel, G. D. and Torrie, J.H. 1980, "P*rinciple and Procedure of statistics*. A Biometrical Approach", (Second Edition), McGraw-Hili Book Company Incorp. New York, .

Uhegbu, F.O. and Ogbuehi, K.J. 2004, "Effect of aqueous crude leaf extract of *Vernonia amygdalina* (Del.) on blood glucose, serum albumin and cholesterol levels in diabetic albino rats", Global Journal of Pure and Applied Science **10**(1) 189 - 194.