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HYPOGLYCEMIC EFFECT OF *TRECULIA AFRICANA* DECNE ROOT BARK IN NORMAL AND ALLOXAN-INDUCED DIABETIC RATS.

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Abstract

The solvent partitioned purified fractions of the hydro-acetone root bark extract of the African breadfruit (*Treculia africana* Decne) were evaluated for hypoglycemic activities in normal and diabetic albino rats. Fasting blood glucose levels were estimated by the use of a glucometer at pre-determined intervals after oral administration of the test extracts/fractions. Results revealed that the test fractions have only a slight effect on blood sugar level of normal rats. On short term and chronic administration in diabetic rats however, diethyl ether-soluble (DEF) and the water-soluble (WSF) fractions significantly reduced the fasting blood sugar levels ($p<0.05$) at differing rates when compared with the control group of animals. The diethyl ether soluble fraction (10 mg kg^{-1} dose level) was found to exhibit the highest activity giving 69.4% reduction in blood sugar level (at 240 hours) which was in comparable range with the reference standard glibenclamide (0.5 mg kg^{-1}) which reduced blood sugar levels by 65.8% below the initial baseline values.

Key words: *Treculia africana*; Moraceae, hypoglycemic activity, alloxanized albino rats.

Introduction

The increasing incidence of diabetes mellitus worldwide is a major health concern especially since there has been a distinct lack of substantial progress in the development of new, effective, safe and inexpensive therapies (Greenfield and Chisholm, 2004). The disease affects well over 15.1 million people in North America, 18.5 million in Europe, 12.6 million in Latin America, 6.6 million in former USSR, 5.3 million in Africa and about 1 million in Oceania (Greenfield and Chisholm, 2004). Although different categories of medication such as insulin, sulphonylurea, biguanides, thiazolidinediones are presently available to treat diabetes (especially non-insulin dependent diabetes, NIDDM), they are however limited in their pharmacokinetic characteristics and ability to manage the disease effectively (Maggs et al., 1998; Misbin et al., 1997). In addition, concerns of physicians over side effects observed with current medication in patients underlines the need for novel approach to treatment.

Ever since the call by WHO for a renewed search and discovery of new medicinal agents from natural sources (WHO, 1980), increasing number of medicinal plants and herbal remedies are being screened for hypoglycemic activity (Anturtikar et al., 1995; Gao, 1989; Ogundipe et al., 2003). Plants with ethno-medicinal and [a]

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nutritive values have the potential to provide important means or leads for a rational treatment and management of a disease like diabetes, the cure of which has eluded physicians and health care providers for centuries.

Treculia africana Decne (Moraceae), commonly known as African breadfruit is a plant food native to tropical West and parts of East Africa. Ethno-medicinally, it is used as a vermifuge, febrifuge galactogogue and laxative (Irvine, 1961). The plant is also an important component of some ancient anti-diabetic recipe used in Western and Middle Belt of Nigeria as our survey among herbalists and number of patients attending Diabetic clinics in the University College Hospital, Ibadan revealed. . Several workers have evaluated the pods of *T. africana* for its nutritive properties (Edet et al., 1985; Akubor and Badifu, 2004), flavonoid, phenolic and polysaccharide content (Oliviera and Santos, 1974; Prista and Alves, 1961; Chukwu et al., 1997). There is however no scientific data available regarding the anti-diabetic effect of this plant.

In continuation of our investigations into the hypoglycemic activities of African plant foods (Ogundipe et al., 2003), the objective of the present study is to evaluate the effect of extracts obtained from of *T. africana* root bark on the blood sugar levels in both diabetic and non-diabetic animal models.

Materials and Methods

Plant Materials

Treculia africana root bark was collected in Mid-July 2004 from the Forest Research Institute of Nigeria (FRIN) compound. The plant sample was subsequently authenticated by Mr O Odewo and a voucher specimen (PCG 038) is deposited in the Pharmacognosy herbarium.

Phytochemical Screening/Extration of plant materials

The presence of the following secondary metabolites in the crude plant and fractions was evaluated using standard procedures: alkaloids, anthraquinones, cardenolides, flavonoids and tannins (Trease and Evans, 1996).

Air-dried and pulverized root bark (250 g) was macerated in 2 L extraction solvent (acetone: water 4:1) for 7 days. The extract was filtered, concentrated to low volume and then partitioned with diethyl-ether. The diethyl ether soluble fraction (DEF) was dried to a viscous residue to give a 1.64 % w/w yield. A portion of the diethyl ether soluble residue (1 g) was suspended in 2% w/v methyl cellulose. The dried water-soluble fraction (WSF) was also reconstituted into a 2% methyl cellulose suspension.

Thin-Layer Chromatography

Analytical: Silica Gel GF_{254} (Merck 0.25 mm thick, activated at 100 ^{o}C before use
Solvent system: Toluene : acetone: chloroform (1.8:2:1.2)
Visualization : UV395, $FeCl_3$

Experimental animals

Adult Wistar albino rats with an average weight of 100 g used in this study were obtained from the animal house of the Department of Biochemistry, University of Ibadan. The animals were kept and maintained under laboratory conditions of temperature, humidity and light and were allowed free access to standard laboratory diet (Ladokun Feeds Ltd, Ibadan) and water *ad libitum* for a week before the evaluations were carried out. All experimental protocols were in compliance with our institutional ethical committee guidelines as well as internationally accepted principles for laboratory animal use and care as found in US guidelines (NIH publication # 85-23, revised in 1985).

Hypoglycemic activity evaluation

Thirty-two albino rats were fasted overnight but allowed free access to water.

The animals were divided into “diabetic” rats and “normal” rats. The “diabetic” rats were made diabetic by a single intravenous injection of freshly prepared alloxan monohydrate solution at a dose of 80 mg kg^{-1}. The diabetic status of the rats were confirmed by the fasting blood glucose levels (180-350 mg/dl) obtained 48 hours after administration and which is maintained throughout the duration of the experiment.

Diabetic rats were divided into four groups I-IV (n=4) while the normal rats were also divided into four groups V-VIII (n = 4) and extracts/ standard reference drugs were administered as follows:

Diabetic rats

Group I - rats in this group received standard drug glibenclamide 0.5 mg kg^{-1} p.o. Group II – rats in this group received (DEF, 10 mg kg^{-1}) p.o. Group III- rats in this group received (WSF, 10 mg kg^{-1}) p.o. Group IV – rats in this group received only the vehicle (2% carboxymethyl cellulose in normal saline)

Normoglycemic rats

Group V- normal rats in this group received standard drug glibenclamide 0.5 mg kg^{-1} p.o Group VI – normal rats in this group received (DEF, 10 mg kg^{-1}) p.o. Group VII- normal rats in this group received (WSF, 10 mg kg^{-1}) p.o. Group VIII – normal rats in this group received only the vehicle (2% carboxymethyl cellulose in normal saline. Fasting blood sugar was monitored at 2-hourly intervals after the administration of extracts. Subsequent administration of test extacts/reference drug were carried out daily for another 6 days after the fasting blood glucose levels have been determined each day. Blood samples were obtained via the tail blood and glucose levels were measured with a glucometer (Reflolux® S), standardized by glucose test strips (code 212). Administration of the extracts was stopped on the 7th day after which the animals were observed for an additional three (3) days. The last fasting blood sugar was recorded on the tenth (10^{th}) day of the experiments.

Statistical analysis

Results are expressed as mean ± sem of four determinations. The significance of the differences between the means of the test and control animals were established by the student's t-test and values lower than 0.05 were considered to be significant.

Results and Discussion

The results of the present study (Table 1) showed that the fractions obtained from the hydro-acetone root extract have good hypoglycemic effect in diabetic rats at a very low dose of 10 mg kg^{-1}. The diethyl ether soluble fraction (DEF) exhibited a 27.1 % sugar lowering effect within the first 4 hours of administration and had its optimal effect (69.4 %) at 240 hours. The standard reference drug (glibenclamide), an oral hypoglycemic agent at a dose of 0.5 mg kg^{-1} gave corresponding figures of 26.4 % and a peak effect of 65.8 % at 240 hours.

Table 1: Effect of *T. africana* extracts on fasting blood glucose level of alloxan-induced diabetic rats (Mean ± SEM) [a]The values in parentheses represent the percentage of decrease in blood glucose level * Significant difference in glucose level when compared with baseline values ($p < 0.05$)

Treatment (p.o)	Blood glucose mmol/l at different hours after treatment[a]						
	Basal value	2 h	4 h	24 h	72 h	144 h	240 h
Glibenclamide 0.5 mg kg^{-1}	215.75±11.49	189.50±12.81 (12.2)	*158.75 ± 10.39 (26.4)	264.25± 6.00	*80.00 ± 6.14 (62.9)	*96.50±10.53 (55.3)	*73.75 ± 5.51 (65..8)
Diethylether extract DEF 10 mg kg^{-1}	193.75± 4.61	*147.25 ± 7.33 (24.0)	*141.25 ± 2.86 (27.1)	*153.25 ± 5.30 (20.9)	*82.00 ± 1.69 (57.7)	*67.75 ±4.30 (65.0)	*59.25 ±2.32 (69.4)
Water soluble extract WSF 10 mg kg^{-1}	305.25 ± 4.38	249.75 ± 4.93 (18.1)	271.00 ± 5.86 (11.2)	*233..75 ± 3.80 (23.4)	*221.00 ± 3.35 (27.6)	*74.25±5.90 (75.7)	*140.25 ± 4.11 (54.1)
Diabetic control (untreated)	238.67 ± 4.09	261.00 ± 3.46	309.70 ± 2.05	349.00 ± 4.02	378.00 ± 4.34	382.00± 8.72	380.00 ± 2.94

A progressive reduction in blood sugar level following repeated dosing of DEF to diabetic rats was observed. Comparing the effect of DEF with glibenclamide, it could be observed that although the lowering effects of the two treatments at 72 h post administration are similar, there was continued reduction in sugar level by DEF at 144 h in contrast to the increment in sugar levels exhibited by the reference group of rats. DEF (10 mg kg^{-1} dose level) therefore showed a better overall sugar lowering effect in diabetic rats than glibenclamide (0.5 mg kg^{-1}) which was chosen as a standard reference drug in this study as it is well known to be one of the most frequently used anti-diabetic drugs (Balazs et al., 2005).

Table 2: Effect of *T. africana* extracts on fasting blood glucose level of normal rats (Mean ± SEM)

Treatment (p.o)	Blood glucose mmol/l at different hours after treatment[a]						
	Basal value	2 h	4 h	24 h	72 h	144 h	240 h
Glibenclamide 0.5 mg kg^{-1}	62.00 ± 4.73	44.25± 6.86	41.00 ± 7.25	65.75 ± 6.69	44.75 ± 6.17	50.25 ± 7.55	62.50± 4.25
Diethylether extract DEF 10 mg kg^{-1}	55.00 ± 2.63	*36.75 ± 5.45	44.75 ± 7.62	60.25 ± 1.50	53.75 ± 7.16	42.75 ± 0.46	57.50 ± 7.51
Water soluble extract WSF 10 mg kg^{-1}	57.25 ± 5.66	47.25 ± 10.28	50.00 ± 8.66	58.5 ± 6.47	64.75 ± 3.12	63.75 ± 10.16	55.25 ± 9.90
Control (untreated)	54.25 ± 4.03	70.00 ± 8.25	71.75 ± 2.47	70.50 ± 3.38	60.25 ± 4.90	53.75 ± 1.49	44.50 ± 4.41

* Significant difference in glucose level when compared with baseline values ($p < 0.05$)

The water soluble fraction (WSF) of the hydro-acetone extract also showed a strong activity, although a cursory observation of Table 1 would give the impression that it is less active than DEF. A careful examination shows that WSF may have some pharmacokinetic advantage over DEF because of the more gradual and controlled decreases in blood sugar level exhibited by it, not only within the first 24 h, but throughout the experimental period. This characteristic may be particularly useful where reduction of incidence of unwanted side effects such as hypoglycemia is a major concern.

It is also of interest to note that the significant reduction of the residual effect of WSF on blood sugar level after the termination of its administration when compared with the residual effects of DEF and the reference glibenclamide, both of which continue to exert high hypoglycemic activity 72 hours after the last doses were administered. One likely implication of this observation is that the clearance of WSF from the system is much faster than that of DEF and glibenclamide. There may also be less tissue accumulation of WSF than that obtained for the other fractions/reference drug..

In healthy normoglycemic rats, the effects of both DEF and WSF are comparable with glibenclamide which produces little or no significant overall reduction in fasting blood sugar levels on repetitive dosing.(Table 2)

Phytochemical and thin layer chromatographic analysis of the plant material showed that the crude extracts and fractions contain mainly, flavonoid-related and anthraquinoid compounds, the characterization of which are subjects of further investigations in our laboratory. Although claims that flavonoids, like glibenclamide, are able to regenerate the damaged beta cells in alloxanised diabetic rats (Chakravarthy et al., 1981; Chakravarthy et al., 1982) have been severally refuted (Sheehan et al., 1983; Kolb et al., 1982), the hypoglycemic effects of *T. africana* extracts in this study may, in part, not be unconnected with the flavonoid and related phenolic component as these classes of secondary metabolites have been shown to be bioactive anti-diabetic principles present in other medicinal plants such as *Bauhinia forticata* Link and *Pterocarpus marsupium* (de Sousa et al., 2004; Manickam et al., 1997).

In conclusion, the data in our present study suggest that *Treculia africana*, a valued food plant growing abundantly in Nigeria, may have beneficial effects in type-1 diabetes mellitus and may serve as source of bioactive molecules for future generation of anti-diabetic drugs. Comprehensive pharmacological and phytochemical investigation is however required to determine the exact mechanism of action of the hypoglycemic activity of the extracts/fractions as well as to isolate and elucidate the structure of the bioactive molecules responsible for the effects observed

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