

## Hypoglycaemic Effect of Crude Ethanol Extract (EE) of the Leaves of *Diaphanathe bidens* [(Afzel. Ex. Sw.) Schltr. 1914] on Diabetic Wister Rats

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### Abstract

*The hypoglycaemic and anti-hyperglycaemic effects of the dried leaves of Diaphanathe bidens (family-Orchidaceae) were evaluated in normoglycaemic and alloxan-induced diabetic male albino rats. Normoglycaemic and alloxan-induced diabetic male rats were treated per os (P.O.) with ethanol extract of Diaphanathe bidens, glibenclamide (positive control) and normal saline (negative control). The serum glucose concentrations were determined by the use of the one-touch blood glucose monitoring system/meter and test strips (life scan Inc. Johnson-Johnson Company, Mulpiter California, USA). The classes of chemical components of the ethanol extract of the leaves of Diaphanathe bidens were determined; alkaloids, carbohydrates, glycosides, reducing sugars, flavonoids, terpenoids, steroids, resins and tannins were found to be present. Acute toxicity test of the extract in mice gave an LD<sub>50</sub> of 1500mg/kg. In normoglycaemic rats, the ethanol extract (EE) of Diaphanathe bidens 500mg and 750mg/kg exhibited 5.42% (P<0.05) and 6.7% (P<0.05) significant reduction of the blood glucose levels respectively within 20h of administration while glibenclamide 600mg/kg showed 48.35% (P<0.05) significant reduction. In alloxan-induced diabetic rats, the ethanol extract (EE) caused 32.70% (P<0.05) reduction in blood glucose concentration. The study showed that the dried crude ethanol extract (EE) of Diaphanathe bidens has significant hypoglycaemic activity in normglycaemic and alloxan-induced diabetic male albino rats.*

**Key words:** Hypoglycaemia, *Diaphanathe bidens*, Diabetes, Glucose concentration

### Introduction

According to the World Health Organization, at least 171 million people worldwide suffer from diabetes mellitus. Its incidence is increasing rapidly, and it is estimated that by the year 2030, this number will double. Diabetes mellitus is a medical disorder characterized by varying or persistent hyperglycaemia (high blood sugar levels) resulting from the defective secretion or action of hormone insulin. There are two predominant forms of diabetes. Type 1 diabetes (previously called juvenile onset diabetes) is characterized by decreased or absent production of insulin. Type 2 diabetes (previously called adult onset diabetes), the more common form is characterized by body tissue resistance to insulin action, though decreased secretion of insulin can also occur. Type 1 diabetes almost always requires insulin injections for survival, whereas type 2 diabetes can often be managed by dietary monitoring, weight reduction, exercise and oral medication. Insulin is used in Type 2 diabetes if oral medication proves ineffective or has intolerable side effects. Most cases of Type 2 diabetes are treated with medication, although about 20% of them may be managed by lifestyle changes alone. Type 1 diabetes is an autoimmune disorder in which the body makes antibodies that attack the insulin-producing cells in the pancreas. Type 1 diabetes was once called juvenile diabetes because it is usually diagnosed in childhood or early adulthood. People with type 1 diabetes must supply insulin by injection. Possible treatment include transplant of a pancreas or beta cells. Whilst under or over-treated diabetes can be extremely dangerous due to abnormal levels of glucose, for most treated diabetic patients the main risks are

from long-term complications including cardiovascular disease, chronic renal failure, retinal damage which can lead to blindness, nerve damage which can lead to erectile dysfunction (impotence), gangrene with risk of amputation of toes, feet and even legs. Serious complications are much less common in people who control their blood sugars well with their lifestyle and medications. Other health problems that accelerate the damaging effects of diabetes should also be addressed; including smoking, elevated cholesterol levels, obesity, high blood pressure and lack of regular exercise.

The non-pharmacological means (diet and exercise) and/or the pharmacological means (insulin and oral hypoglycaemics) may be used in the management of diabetes mellitus. The obvious limitations of these management methods necessitated a search for alternatives among the arsenal of herbal principles available to man. It was in this light that the World Health Assembly in 1989, adopted among its resolutions, the support of national traditional medicine programme, drawing attention to herbal medicines as being of great importance to the health of individuals and communities. Records from ancient Egypt, Assyria, china and India show that the use of plants for medicinal principles extends back to earliest recorded history (Trease and Evans, 1996).

### Materials and Methods

**Plant material:** *Diaphanathe bidens* [(Afzel.ex.sw.) Schltr. 1914], subspecies *bidens* belongs to the family Orchidaceae. It grows in Central and West Africa at an elevation of 350 to 1300metres above sea level as a medium sized

plant, epiphyte with elongate stems carrying numerous ovate or elliptic, unequally and acutely bilobed apical leaves that blooms in the dry season (Guy, 2004). *Diaphanthe bidens* plant was collected from the environs of Nsukka Local Government Area of Enugu State and was identified by Mr. A.O. Ozioko of Bioresources Development and Conservation Programme (BDCP), Nsukka (formerly Herbarium section, Department of Botany, University of Nigeria, Nsukka).

**Extraction procedure:** The leaves of the plant was chopped into tiny pieces, dried under room temperature for seven days and pulverized with mortar and its pestle into a coarse powder, then macerated in 1000ml of analytical ethanol and left to stand for 24h with occasional stir. Filtration was carried out and the filtrate was then concentrated by exposing it under the fan until all the ethanol evaporated to obtain the ethanol extract as solid residue. The ethanol extract weighed 65g, giving a 13% yield from the 500g weight of the dried leaves started with.

**Animals:** The rats used for this study were male wister albino rats of about 8-10 weeks old with average weight of about 100-200g. The mice used for the LD<sub>50</sub> were about 8-10 weeks old and weighed between 13-12g. They were obtained from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The Animals were stabilized in cages and fed with pelletized Guinea Grower's mash from Bendel Feed and Flourmill limited, Nigeria for 7days with free access to water before and throughout duration of the experiments.

To evaluate the hypoglycaemic effect of the EE in normal rats that were non-diabetic.

Twenty normoglycaemic rats having sugar concentrations of 54-61mg/dl after 12h fasting were allowed access to water before and throughout the duration of experiment. They were then divided into four groups of five rats each.

Group I (EE): Normal rats (non-diabetic) given 500mg/kg b.w ethanol leaf extract.

Group II (EE): Normal rats (non-diabetic) given 750mg/kg b.w. ethanol leaf extract.

Group III (NS): Normal rats (non-diabetic) given 0.85% w/v of normal saline.

Group IV (PC 600): Normal rats (non-diabetic) given 600mg/kg b.w. of glibenclamide as the standard hypoglycaemic agent.

All the administrations were through oral routes. At time interval (0, 1, 4, 8, 12, 16, 20 and 24 h) after treatment. Blood samples were collected from the tail (tail nipping) of the rats and blood sugar concentration determined using a glucometer.

To evaluate the hypoglycaemic effect of EE using hyperglycaemic rats, another group of twenty-five rats having sugar concentrations of 50-74mg/dl after 12h fasting were injected intraperitoneally (i.p.) with 200mg/kg body weight of freshly prepared alloxan monohydrate (sigma, USA) in normal saline. The animals were fed for seven days with Bendel Feed and Flour Mill Limited Pelletized Guinea Grower's mash. On day 8, the 20 surviving diabetic rats were divided into three groups of five animals each.

Group I (EE): Diabetic rats given 750mg/kg b.w. ethanol leaf extract.

Group II (NS): Normal rats (non-diabetic) given 0.85% w/v of normal saline.

Group III (PC600): Diabetic rats given 600mg/kg b.w. of glibenclamide as the standard hypoglycaemic agent.

After seven days of treatment blood samples were collected from the tail of surviving rats at intervals of 0, 1, 4, 8, 12, 20, 24hours and the blood glucose concentration determined using a glucometer.

Determination of LD<sub>50</sub> of the extract:

Medium lethal dose (LD<sub>50</sub>) is the log dose of a drug that kills 50% of adult albino mice (2 males and 2 females per group) were used for the experiment. The extract, dissolved in normal saline, were injected intraperitoneally in doses of 200, 400, 500, 1000 and 1500mg/kg body weight in groups 1 to 5 respectively. The animals were fed with the normal rat feed and water. The number of animals dead within 24 hours after injection was recorded for each group. Log doses of the extract were plotted on a graph against profits of the percentage dead, and LD<sub>50</sub> extrapolated according to the method of Miller and Tainter (1944).

## Results

The results presented are the mean values  $\pm$  S.D and statistical significance between treated and a control group was determined using students't-test. P<0.05 was considered significant (Woodson, 1987).

## Discussion

The increase in number of diabetic patients in recent times has motivated scientists to look for alternative methods for curing diabetes (Adeghate, 1999). In the present study, the hypoglycaemic effect of the ethanol extract of *Diaphanthe bidens* was tested on normoglycaemic as well as alloxan-diabetic rat models.

The result of the phytochemical tests revealed the presence of alkaloids, carbohydrates, glycosides, reducing sugar, flavonoids, terpenoids, steroids, resins and tannins in the dried crude ethanol extract. Proteins saponin, fats and oil were however absent. The alkloids and flavonoids are said to have medicinal properties in animals (Livingstone et al, 1997). However, high concentrations of these substances are toxic and may impair body metabolism (Weislaw et al; 1999).

Acute toxicity test of the ethanol extract on mice gave an LD<sub>50</sub> of 1500mg/kg, which indicates that the extract is relatively safe for consumption.

The results of the hypoglycaemic effect of the ethanol extract of *D. bidens* is shown in Table 1 and 2. a dose dependent reduction in fasting blood glucose concentrations of the treated rats was observed. In normaglycaemic rats (Table 1), the ethanol extract of *D. bidens* (500 and 750mg/kg exhibited 5.4% (P<0.05) and 6.70% (P<0.05) reduction respectively.

**Table 1: Effect of the ethanol extract of *D. bidens* on normoglycaemic rats**

Groups	<i>D. bidens</i>	Fasting Blood Glucose (mg/100ml) Time (Hrs) after Treatment						% Max. Reduction	
		0	1	4	8	12	16		20
GRP I	Normal Saline (0.85%)	53.50 ± 4.3	52.50 ± 8.9	51.25 ± 6.4	49.75 ± 3.4	51.00 ± 10.1	52.80 ± 13.3	51.50 ± 9.6	0.00
GRP II	Ethanol extract (500mg/kg)	60.00 ± 3.5	53.00 ± 5.1	59.00 ± 5.0	54.50 ± 5.0	47.76 ± 4.3	46.00 ± 6.2	56.75 ± 2.6	5.42
GRP III	Ethanol extract (750mg/kg)	52.25 ± 4.6	55.25 ± 7.4	54.00 ± 7.5	52.25 ± 9.8	51.50 ± 8.6	50.50 ± 12.0	48.75 ± 10.5	6.70
GRP IV	Glibenclamide (600mg/kg)	60.50 ± 9.6	44.00 ± 5.2	36.50 ± 6.9	33.25 ± 10.9	32.00 ± 1.2	31.25 ± 1.9	55.75 ± 11.3	48.35

**Table 2: Effect of the ethanol extraction of *D. bidens* on alloxan-induced rats**

Groups	<i>D. bidens</i>	Fasting Blood Glucose (mg/100ml) Time (Hrs) after Treatment						% Max. Reduction	
		0	1	4	8	12	16		20
GRP I	Normal Saline (0.85%)	61.25 ± 4.8	61.50 ± 9.0	61.25 ± 5.0	61.75 ± 17.0	61.75 ± 8.0	61.25 ± 4.6	61.50 ± 7.8	0.00
GRP II	Ethanol extract (750mg/kg)	208.75 ± 35.6	382.00 ± 132.8	313.75 ± 69.5	255.50 ± 103.8	152.00 ± 40.9	140.50 ± 4.6	151.75 ± 2.7	32.70
GRP III	Glibenclamide (600mg/kg)	182.20 ± 30.6	164.56 ± 37.4	144.00 ± 44.3	83.25 ± 9.2	76.25 ± 5.3	69.75 ± 4.3	47.40 ± 4.6	73.98

In alloxan-induced diabetic rats (Table 2) the ethanol extract of *D. bidens* (750mg/kg) exhibited a significant 32.70% ( $P < 0.05$ ) reduction of blood glucose concentration within 20h of administration, while glibenclamide (60mg/kg) caused 73.99% reduction. The extract produced more marked significant reduction in blood sugar concentration in the alloxan-induced diabetic rats than in the normoglycaemic rats.

As our main objective was to establish the presence or absence of hypoglycaemic effect on the dried crude extract, the present result showing significant hypoglycaemic activity in normoglycaemic and anti-hyperglycaemic activity in alloxan-induced diabetic rats confirm hypoglycaemic effect and validates further its use in traditional medicine. In normoglycaemic rats the hypoglycaemic effect of 500mg/kg of the extract was statistically comparable to that of 600mg/kg of glibenclamide. In alloxan-diabetic rats, the reduction in blood glucose concentration within 20h of treatment with the ethanol extract (750mg/kg) was also significant ( $P < 0.05$ ) and was comparable to that produced by 600mg/kg of the standard drug, glibenclamide. The studies showed that the dried crude ethanol extract of *D. bidens* has significant ( $P < 0.05$ ) dose-dependent hypoglycaemic and anti-hyperglycaemic activities in normoglycaemic and alloxan-induced diabetic male albino rats. The mechanism of action of this effect is yet to be established. Alloxan is known to induce diabetes due to direct pancreatic Bcell cytotoxicity (Zarrow et al, 1964). Following from this it could be adduced that the observed hypoglycaemic effect of the ethanol extract in the non-diabetic and alloxan-induced diabetic rats seem to lend credence to pancreatic and peripheral mechanism of action.

In conclusion, the crude ethanol extract of dried leaves of *D. bidens* show dose-dependent significant ( $P < 0.05$ ) hypoglycaemic activity in non-diabetic and alloxan-induced diabetic rats.

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