REPORT

HYPOGLYCAEMIC ACTIVITY OF ETHYL ACETATE FRACTION OF THE LEAF EXTRACT OF *TELFAIRIA OCCIDENTALIS*

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ABSTRACT

The leaf of *Telfairia occidentalis* has been found to possess hypoglycemic or antihyperglycemic effect. The hypoglycemic principle of the leaf is yet to be isolated. The aim of this study is to evaluate the hypoglycemic activity of some fractions of ethanolic leaf extract of *Telfairia occidentalis* in rat as a step toward activity directed isolation of the hypoglycemic component. Ethanolic leaf extract was successively extracted with ethyl acetate, butanol and ethanol to obtain ethyl acetate, butanol and ethanol fractions (I-III). The residue was taken as fraction IV. 250 mg/kg of the various extracts were orally administered to normoglycaemic and alloxan-induced diabetic albino rats. Blood glucose concentration was evaluated at 0, 1, 2 and 4 hours after treatment with One Touch glucometer. None of the fractions reduced glucose concentration in the normoglycaemic rats, while only ethyl acetate fraction lowered glucose concentration significantly at 2 and 4 hours (49.7 and 39.0%) compared to control value of 74.9 and 69.7%, respectively, in the diabetic rats. The results showed that the hypoglycemic component of the ethanolic leaf extract of the plant is contained in the ethyl acetate fraction.

Keywords: *Telfairia occidentalis*, hypoglycemic, ethyl acetate fraction.

INTRODUCTION

Telfairia occidentalis Hook F (cucurbitaceae) popularly known as fluted pumpkin is widely cultivated for its palatable and nutritious leaves which are used mainly as vegetables (Sanni, 1982). When compared with other tropical vegetables, the leaves have high nutritive value. Its protein content (21%) is higher than that of water leaf (Talinum), Okra (Abelmoschus), cassava leaves, and other commonly used leafy vegetables (Bosa *et al*., 1983). The leaves are rich in vitamins and minerals such as Ca, P and Fe (Whitaker and Davies, 1979; Jeffrey, 1980; Iwu, 1983).

Researchers are gradually focusing attention on the medicinal value of the plant. The plant is known to be helpful in the protection against atherosclerotic cardiovascular disease (Odoemena and Onyeneke, 1988); stimulation of bone marrow to produce blood cells and maintenance of body resistance to infection and in remedy of all cases of anemia (Oyolu, 1978; Ajayi *et al*., 2000 and Alada, 2000); treatment of hypercholesterolemia, liver problems and impaired immune system (Eseyin *et al*., 2005a; Oboh, 2005); treatment of malaria (Okokon *et al*., 2007); management of diabetes mellitus (Aderibigbe *et al*., 1999; Eseyin *et al*., 2000; Nwozo *et al*., 2004; Eseyin *et al*., 2005b; Eseyin *et al*., 2007). This work was undertaken to determine which fraction contained the hypoglycemic agents of the leaf.

MATERIALS AND METHODS

Plant collection and Identification

The leaves of *Telfairia occidentalis* was collected from the medicinal plant garden of the Faculty of Pharmacy, University of Uyo, Nigeria in January 2007 and identified in the same Faculty.

Preparation of extract

The fresh leaves collected were washed with water. The water was allowed to drain off. 2kg of the leaves were chopped into smaller bits and ground with a mortar and pestle. The leaf material was macerated with 4litres of 96% ethanol for three days at room temperature with periodic shaking. On the third day the extract obtained was filtered and concentrated using a rotary evaporator. The concentrated extract was dried in a desiccator containing silica gel (self- indicating) to give a yield of 3.5% w/w. 20g of the leaf extract was successively extracted with 250ml of ethyl acetate, butanol, and ethanol to obtain ethyl acetate (I), butanol (II) and ethanol (III) fractions. The residue obtained was also treated as a fraction.

Animals

White albino rats (Wistar strain) of both sexes (205±35g) purchased from the University of Uyo animal house were used. The rats had free access to standard chow and water. They were kept under standard laboratory conditions at room temperature and were exposed to 12 hours light and

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12 hours dark periods. The US guidelines (NIH publication No. 85-23, revised in 1985) were followed in the handling of animals.

Induction of diabetes

White albino rats were fasted overnight. Freshly prepared 5% aqueous solution of alloxan monohydrate was injected intraperitoneally to the rats at a dose of 150 mg/kg and left for 5 days. At the end of the 5th day the blood glucose concentration of each rat was evaluated. Rats with blood glucose concentration of 13.0mmole/L and above were considered diabetic.

Administration of Extracts

Effect of different fractions on normoglycaemic rats

Each of the four fractions (I, II, III and IV) was orally administered to separate groups of overnight fasted normoglycaemic rats (five rats per group) at a dose of 250 mg/kg. Control group received water only. Blood glucose was evaluated at 0, 1, 2 and 4 hours after administration.

Effect of different fractions on diabetic rats

Each of the four fractions (I, II, III and IV) was orally administered to separate groups of overnight fasted alloxan induced diabetic rats (five rats per group) at a dose of 250 mg/kg. Control group received water only. Blood glucose was evaluated at 0, 1, 2 and 4 hours after administration.

Estimation of blood glucose concentration

Blood collected from the tail vein of the rats was analyzed for glucose using One Touch glucometer (Lifescan, U.S.A.).

STATISTICAL ANALYSIS

All data were expressed as the mean SEM. Student's t test for unpaired samples was performed using Graphpad prism (Graphpad software, version 3.02). A value of $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Results obtained are shown in tables 1 and 2. The dose of 250 mg/kg used was based on the fact that 250 mg/kg of the leaf extract exhibited hypoglycemic activity in an earlier work done by the authors (Eseyin *et al.*, 2005b). In normoglycaemic rats none of the fractions reduced blood

Table 1: Effect of different fractions of the leaf extract (250mg/kg) on the blood glucose levels of normoglycaemic rats

	0 hour	1 hour	2 hours	4 hours
Control	2.20±0.33 (100)	2.30±0.42 (104.5)	2.28±0.18 (106.0)	1.70±0.21 (79.2)
Ethyl acetate fraction	2.70±0.29 (100)	2.46±0.27 (90.7)	2.32±0.43 (86.1)	2.44±0.32 (90.9)
Butanol fraction	2.82±0.32 (100)	2.96±0.34 (104.9)	2.72±0.55 (97.3)	2.68±0.43 (95.3)
Ethanol fraction	3.04±0.39 (100)	2.96±0.28 (99.9)	2.46±0.40 (82.7)	2.82±0.34 (92.3)
Residue	2.42±0.22 (100)	3.04±0.44 (127.0)	2.82±0.38 (116.4)	2.32±0.29 (96.9)

Mean ± SEM, *Significantly different from control, $p < 0.05$. Figures in parenthesis are percent of 0 hour value.

Table 2: Effect of different fractions of the leaf extract (250mg/kg) on the blood glucose levels of diabetic rats

	0 hour	1 hour	2 hours	4 hours
Control	14.6±3.33 (100)	11.5±4.44 (79.4)	10.9±4.14 (74.9)	10.2±2.53 (69.7)
Ethyl acetate fraction	13.9±3.91 (100)	9.30±3.09 (66.9)	6.9±2.76 (49.7)*	5.4±1.89 (39.0)*
Butanol fraction	13.4±2.99 (100)	8.9±3.22 (66.2)	7.8±2.88 (58.0)	7.5±2.72 (56.7)
Ethanol fraction	14.4±5.01 (100)	10.9±3.76 (75.8)	10.7±3.44 (74.5)	10.8±4.11 (75.1)
Residue	13.6±5.12 (100)	9.1±2.96 (66.9)	8.5±2.33 (62.2)	6.3±2.85 (46.4)

Mean ± SEM, *Significantly different from control, $p < 0.05$. Figures in parenthesis is percent of 0 hour value

glucose concentration significantly (table 1). While in the alloxan induced diabetic rats only ethyl acetate fraction (fraction I) had significant blood glucose lowering effect at 2 and 4 hours after treatment (table 2). In the normoglycaemic rats, the pancreas is fully functional and hypoglycemic agents which stimulate the beta cell of the pancreas to secrete or release insulin will reduce blood glucose concentration. But in alloxan induced diabetic rats, the pancreatic islet beta cells are damaged thus causing insulin dependent diabetes mellitus (IDDM or type 1 diabetes). This occurs because of selective uptake of alloxan by the beta cell of the pancreas due to its structural similarity to glucose as well as the beta cell's high efficient uptake mechanism. In this case an oral hypoglycaemic agent which functions only through the pancreatic beta cells will have no hypoglycemic effect (Hikino, 1989; Huralikuppi, 1991).

Therefore, since the ethyl acetate fraction lowered glucose concentration in the alloxan diabetic and not in the normoglycaemic rats, its hypoglycemic effect is extra pancreatic. In an unpublished work by the authors, column chromatographic analysis of the ethyl acetate fraction yielded three components with Rf values of 0.88, 0.22 and 0.99 all of which gave positive color reactions when sprayed with Dragendorff and ferric chloride solution and negative result with vanillin: concentrated sulphuric acid mixture.

CONCLUSION

It could be concluded from the results of this work that the hypoglycemic constituent of the leaf extract is contained in the ethyl acetate fraction and may be a non-polar phenolic alkaloid.

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