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Foliar Epidermal Morphology and Anti-Diabetic Property of *Andrographis paniculata* (Burm. f.) Wall ex. Nees

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Abstract

The leaf epidermal features and anti diabetic property of *Andrographis paniculata* were investigated. The epidermal cells are polygonal to irregular with curved to undulate anticlinal walls on both surfaces of the leaf. The leaf is hypostomatic with stomata restricted to the abaxial surface. Stomata type is axillocytic. The epidermal cells contain deposits of crystal oxalates while cystoliths are found in a few cells. The leaf extract was evaluated for hypoglycemic effects. There was significant hypoglycemic effect in alloxan induced diabetic rats following administration of the leaf aqueous extract. The result shows that the level of fasting blood sugar was dependent on the previous level of basal blood sugar in the four groups of rats used for this study. Treatment of the rats with *A. paniculata* showed a dose dependent relationship with the time it took for the blood of the rats to return to the basal blood sugar levels. The study supports the use of the plant in the management and treatment of diabetes.

Key words: *Andrographis paniculata*; diabetes mellitus, antidiabetic properties, foliar epidermis.

Introduction

Andrographis paniculata (Burm.f.)Wall ex Nees is a small herbaceous plant of about 30-60m high. It is a member of the family Acanthaceae. *A. paniculata* is a native of India or Sri Lanka but now introduced to Nigeria and widely cultivated for its medicinal properties in south western Nigeria. The extract from the leaves and the roots is traditionally and widely used to treat various diseases such as cancer [Kumar *et al.*, 2004; Pajagpal *et al.*, 2003], upper respiratory tract infections [Poolsup *et al.*, 2004; Coon and Ernst, 2004], filariasis [Dutta and Sukul, 1982] and snakebites [Martz, 1992]. *A. paniculata* possesses hypoglycemic properties. An aqueous extract of *A. paniculata* has been shown to have hepatoprotective effects against carbon tetrachloride induced liver damage [Rana and Avadhoot, 1991]. The hypotensive activity of an aqueous extract of the plant has been demonstrated in rats [Zhang and Tan, 1996].

The plant is known to be beneficial following extracorporeal shock lithotripsy [Muangman *et al.*, 1995]. The leaf extract of *A. paniculata* possesses antiplatelet aggregation [Amroyan *et al.*, 1999],

antifertility effect [Zoha *et al.*, 1989] and uterus relaxation [Burgos *et al.*, 2001]. The main bioactive component of the plant is known to be a labdane diterpenoid called Andrographolide (Coon and Ernst, 2004). This study was undertaken to obtain information on the epidermal features of the leaf and to assess the hypoglycemic effect of *A. paniculata* leaf extract based on ancient reports of this property.

Materials and Methods

Plant material

Samples of *A. paniculata* were collected from Kabba, Kabba Local Government Area in Kogi State of Nigeria and the Botanical Nursery of the Department of Botany and Microbiology, University of Ibadan, Ibadan, Nigeria. The plant materials were authenticated at the Herbarium of the Department of Botany and Microbiology, University of Ibadan, Nigeria (UIH) where voucher specimens were also deposited.

Epidermal morphology

Fresh leaves were obtained from the nursery of the Department of Botany and Microbiology, University of Ibadan, Ibadan, Nigeria while the dry leaves from the specimens from Kabba, were boiled in water for about five minutes to revive the cells. Methods of epidermal preparation follow Ayodele and Olowokudejo (1997; 2006). Descriptive terminology is according to Dilcher (1974).

Preparation of the extract

Leaves of *Andrographis paniculata* were collected early in the morning at about 9am. These were air dried at room temperature and later ground into powder form with the aid of an industrial blender. An aqueous extract was obtained by soaking 1g of the powdered leaves in 100ml of distilled water to produce 10mg/ml of aqueous extract.

Experimental animals

Thirty healthy Wistar rats (175-250g) maintained under normal or standard conditions were obtained from the animal house, Department of Veterinary Physiology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan. Animal feeds were obtained from Caps Feed Mill Limited, Ibadan, Nigeria.

Induction of diabetes mellitus in the rats with intramuscular alloxan.

Ten percent alloxan dissolved in physiological saline was used to induce diabetes mellitus in the rats. Ten grammes of fresh sample of alloxan was weighed on a Mettler's balance and dissolved in 100ml of 0.15M physiological saline solutions to produce 10% alloxan. The dose administered was a standard dose of 100mg/kg weight of the rats. The exact volume of the drug administered was obtained using the general formula:

$$\text{Volume to be administered} = \frac{\text{Weight of animal (g)} \times \text{Dose of drug (mg/kg)}}{1000 \times \text{Concentration of drug (mg/ml)}}$$

The rats were divided into six treatment groups of five rats per group. The first four groups of rats were given intramuscular injection of 100mg/kg alloxan and Fasting Blood Sugar (FBS) was checked

after 48 hours. The rats with fasting blood sugar between 100 – 500mg/dl were considered to be diabetic.

Effect of plant extract on the diabetic rats

The diabetic rats in each of the four groups were subsequently treated with different doses of (50mg/kg, 100mg/kg, 200mg/kg and 400mg/kg) of the plant extract twice daily for two weeks. This was administered orally with the use of oral canula. The fasting blood sugar was checked every two days by cutting a bit of the rats' tails for a drop of blood and dropping the blood on a specified space on the glucometer and the reading was recorded. The fifth group (nondiabetic, not injected with alloxan had 400mg/kg of *A. paniculata* administered into them and the fasting blood sugar was recorded every two days for two weeks. The sixth group (control group) was fed with only feeds with clean water daily and their fasting blood sugar observed every two days and compared with the fasting blood sugar of the fifth group.

Statistical analysis

The results are expressed as MEAN±SEM. The student's t test was used to determine the significance of difference between control and treated groups. The level of significance was taken as $p < 0.05$.

Results and Discussion

The results of this study are summarized in Figures 1- 3 and Tables 1-3. Figure 1 shows the habit *A. paniculata* as grown in the nursery of the Department of Botany and Microbiology, University of Ibadan, Ibadan, Nigeria, while figures 2 and 3 are photomicrographs of the adaxial and abaxial epidermis of the leaf of the plant. The epidermal cells are polygonal to irregular with curved to undulate anticlinal walls on both surfaces of the leaf (Figs. 2 and 3). The leaf is hypostomatic with stomata restricted to the abaxial surface. Stomata type is axillocytic (Fig.3) with one cell nearly enclosing both guard cells except for one free pole which is covered by two cells with a common anticlinal wall extending from the pole parallel to the long axis of the guard cells. The epidermal cells contain deposits of crystal oxalates while cystoliths are found in a few cells (Figs. 2 and 3).

Table 1 shows the mean basal blood sugar levels of the four groups of rats and the mean fasting blood sugar levels after two days as a result of the induction of diabetes mellitus by intramuscular administration of alloxan. The result shows that the level of fasting blood sugar was dependent on the previous level of basal blood sugar in the four groups of rats. Treatment of the rats with *A. paniculata* showed a dose dependent relationship with the time it took for the blood of the rats to return to the basal blood sugar levels (Table 2). The maximum time was observed in Group 1 rats (i.e. those treated with 50mg/kg) which was more than 14 days. This was followed by Groups 2 and 3 (100 and 200mg/kg respectively). The shortest time of treatment was observed in the Group 4, those treated with the highest dose (400mg/kg).

The administration of *A. paniculata* in normal rats showed that at the end of the two weeks, the mean fasting blood sugar had reduced from 51.4mg/dl to 42.0mg/dl (Table 3). In summary, the results of this study support the hypoglycemic properties of *Andrographis paniculata* as well as confirm the blood sugar reducing.

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Table 1. The mean values of Fasting Blood Sugar (mg/dl) of the four groups of rats after injection of alloxan.

Groups	Basal Blood Sugar (mg/dl)	Fasting Blood Sugar (mg/dl)
Group1	49.2	195.6
Group 2	49.0	258.0
Group 3	47.8	288.0
Group 4	54.4	342.4

Table 2. The effects of administration of different doses of different doses of *A. paniculata* in various groups of diabetic rats on mean values of Fasting Blood Sugar (mg/dl) taken on alternate days.

Alternate days that Fasting Blood Sugar levels were taken

Groups	2nd	4th	6 th	8th	10th	12th	14th	Mean
Group1 (50mg/kg)	179.6	181.8	149.4	119.2	110.0	80.4	68.4	126.97
Group 2 (100mg/kg)	143.8	117.4	118.8	85.2	60.0	51.8	45.0	88.97
Group 3 (200mg/kg)	204.0	126.0	51.0	49.0	46.6	46.6	45.2	81.22
Group 4 (400mg/kg)	302.2	75.8	62.8	59.0	49.2	43.2	42.8	90.7

Table 3. The mean values of Fasting Blood Sugar (mg/dl) of Group 5 rats and Group 6 (Control group) rats taken on alternate days.

Alternate days that Fasting Blood Sugar levels were taken

Groups	2nd	4th	6th	8th	10th	12th	14th	Mean
Group 5 (No	51.4	60.6	52.0	45.2	42.6	42.6	42.0	48.05

alloxan, but extract at 400mg/kg)								
Group 6 (Control, no treatment)	40.2	50.0	40.0	38.0	38.2	40.6	40.0	40.00



Fig.1: Photograph showing the habit of *Andrographis paniculata*.



Fig. 2: Adaxial epidermal surface of *A. paniculata*.



Fig. 3: Abaxial epidermal surface of *A. paniculata*.