

## Evaluation of antidiabetic activity and histological study of *Cyperus kyllinga* Endl. roots

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Diabetes mellitus is a heterogeneous metabolic disease characterized by altered carbohydrate, lipid and protein metabolism. So many traditional herbs are being used by diabetic patients to control the disease. But very few studies have been performed to investigate the efficacy of these herbs clinically. In the present study, an attempt has been made to investigate the antidiabetic activity of roots of *Cyperus kyllinga* Endl. (Family—Cyperaceae) and also with alcoholic extract of its roots in diabetic mice. The crude roots at (570 mg/kg, orally) and the extract of the roots at (120 mg/kg, orally) showed significant antidiabetic activity. Histological study also showed significant result.

**Key words:** Albino mice, Antidiabetic, *Cyperus kyllinga*, Cyperaceae, Diabetes, Hyperglycemia.

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### Introduction

Hyperglycemia and hyperlipidemia are two important characters of diabetes mellitus, an endocrine disorder based disease. In modern medicine, no satisfactory effective therapy is still available to cure diabetes mellitus<sup>1</sup>. Though pharmaceutical drugs like sulfonylureas and biguanides are used for the treatment of diabetes but these are either too expensive or have undesirable side effects or contraindications<sup>2</sup>. Insulin therapy affords effective glycemic control, yet its demerits are ineffectiveness through an oral administration, short half life, requirement of constant refrigeration of the drug and in the event of excess dosage results fatal hypoglycemia that limits its uses. From various reasons, in recent years, traditional and complementary medicine have been an upsurge in its popularity for the treatment of different diseases as herbal drugs are generally without toxic effect<sup>3,4</sup>.

Diabetes is a world's largest endocrine disease involving metabolism disorders of carbohydrate, fat and protein<sup>5</sup>. According to WHO projection, the prevalence of diabetes is likely to increase by 35%<sup>(Refs 6-8)</sup>. Currently there are over 150 million

diabetics world-wide and this is likely to increase to 300 million or more by the year 2025<sup>(Refs 5-9)</sup>. Statistical projection about India suggests that the number of Diabetics will rise from 15 million in 1995 to 57 million in the year 2025, making the country with the highest number of Diabetics in the world<sup>5</sup>. Therefore it is necessary to look for new solutions to manage this health problem. Although many drugs and interventions are available to manage Diabetes, in most instances these are expensive (like Insulin, Thiazolidinediones) for a developing country like India and have adverse effects (like hypoglycemia)<sup>8</sup>. India is a country with a vast reserve of natural resources and a rich history of traditional medicines.

*Cyperus kyllinga* Endl., of Cyperaceae family is common throughout India. It is also known as *Nirvishaa*, *Nirbishi* and *Mustaa*. The root is used as diuretic (in polyuria), demulcent, refrigerant and antipyretic. It is prescribed for fistula, pustules, tumours, measles, diarrhoea and other intestinal affections. Traces of hydrocyanic acid are reported to be present in the root, stems and nutlets. Rhizome creeping; culms erect, 20-30 cm high, leafy at the base only; leaves narrow linear, flat, scabrid towards the points; involucre 3-4-leaved, spreading. Heads solitary, globose, pale; spikelets compressed, 1-flowered; two lower glumes minute, two upper nearly equal, ovate-lanceolate, sharply keeled, mucronate,

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about 7-nerved; stamens 2; nut broadly ovate, finely punctate, much shorter than the glumes<sup>13,14</sup>.

In the present study we have selected this species whose roots are being used as folklore medicine for many diseases which may control the disease with less or no side effect. The present aim of this work is to explore the scientific basis of the utility of the root extract of *C. kyllinga* for correction of hyperglycemia in diabetes.

## Materials and Methods

### Plant material

Plants of *C. kyllinga* were collected from Bahargram, Panskura, Purba Midnapure, West Bengal, India. Botanical Survey of India confirmed the botanical identity as *Kyllinga nemoralis* (J. R. Forest & G. Forest.) Dandy ex Hutch & Daiziel (= *Cyperus kyllinga* Endl.)<sup>15</sup>. Alloxan monohydrate (Loba.chemical) was obtained from Chemico, West Bengal. All the other chemicals used for the study were of analytical grade.

### Preparation of the extract

The collected roots were shade dried, coarsely powdered and the powder was defatted with petroleum-ether (60-80°C) and later extracted using methanol. After extraction, petroleum-ether and methanol was recovered by distillation. The solvent free extract collected separately and stored for further studies.

### Preparation of treatment diet

Fresh roots of *C. kyllinga* were separated. Roots were dried under shade at room temperature (less than 30°C). After complete drying, roots are grinded into fine powder using a domestic electric grinder. Then extract with methanol and methanolic extract was added to carboxy-methyl-cellulose (CMC) solution (500 mg powder was added to 80 mL water) to get a semi-solid consistency. Then this semi-solid solution is used as diet.

### Animal used

Swiss adult Albino mice were employed under standard laboratory conditions (temperature 24-28°C, relative humidity 60-70%, normal light-dark cycle) in the study. All animals used for the study were obtained from the Animal House of College after getting approval from Institute Ethics Committee. Albino mice (both sex) with body weight ranging from (20 to 40 g) were used for study.

### Preparation of diabetic animals

Mice were made diabetic by a single administration of intra-peritoneal injection (180 mg/kg body wt) of Alloxan monohydrate. Since alloxan could evoke fatal hypoglycemia as a result of massive insulin release, mice were treated with 3 mL 20% glucose solution I.P, 6 hours after alloxan treatment. The mice were then kept for next 24 hours with free access to 5% glucose solution to prevent hypoglycemia<sup>10</sup>. Three days after administration of diabetogenic agent or alloxan monohydrate, blood glucose in fasting condition was determined. Mice having the hyperglycemia symptom selected for experiment.

### Sample collection

Blood samples were collected from tail vein of mice by using needle and syringe.

### Toxicity (LD<sub>50</sub>) evaluation in mice

Albino mice (both sex), 6-8 weeks old with average weight of 25-30 g were used for the study. The methanolic extract was tested for the toxicity in mice. To determine the toxicity, a single oral administration of the methanolic extract of *C. kyllinga* in different doses (500, 1000, 1700 and 1800 and 2000 mg/kg) were administrated to different groups of mice control group received Tween 80. Mortality and general behaviour of the animals were observed periodically for 48 hours. The animals were observed continuously for the initial 4 h followed by 6, 24 and 48 h after drug administration. The parameters observed were hypersensitivity, sedation, respiratory rate and convulsion<sup>16</sup>.

### Experimental design for antihyperglycemic effect

Mice were divided into three groups- Normal Control (NC), Diabetic Control (DC), and Treated Group.

1. To the mice in NC group, were given food and water orally.
2. To the mice in DC group (untreated), were given only CMC (carboxy methyl cellulose) suspension orally.
3. To the Treated Group diabetic mice were given methanolic extract (120 mg/kg) with CMC suspension.

The drug was administered orally for 7 consecutive days. Blood drop was collected from tail foe glucose estimation in fasting condition. The blood glucose level estimated by Accu-Chek Sensor (blood glucose meter) [Roche].

### Histological study

In the histological study on the last day of the experiment the mice were sacrificed and the pancreas

was removed. The pancreas was stored in Bruin's solution and histological slide were prepared and observed under microscope.

#### Statistical analysis

Results are expressed as mean $\pm$ SEM; the significance of the differences between the means of tests and control studies was established by t-test and ANOVA. The P-values less than 0.05 were considered statistically significant.

### Result and Discussion

#### Toxicity (LD<sub>50</sub>) evaluation in mice

No hypersensitivity, sedation, respiratory rate and convulsion changes were observed. The primary acute toxicity studies revealed no visible sign or symptoms of toxicity of the *C. kyllingia* in normal mice at the dose 120 mg/kg. Therefore, the present studies have substantiated the folklore use of *C. kyllingia* for routine treatment of DM and shows that there was no mortality up to dose 1500 mg/kg. The LD<sub>50</sub> value of methanolic extract was found to be 1758.28 mg/kg body weight.

#### Antihyperglycemic studies in Alloxanized (180 mg/kg) mice

The basal blood glucose levels of all groups were statistically not different from each other. Three days after Alloxan administration, blood glucose values were near about 2 folds higher in all the group comparison to NC and were not statistically different from each other. Value of blood glucose level decreased in the entire treatment group but remained stable in diabetic controls<sup>11</sup>. The data are summarized in the Table 1 and Fig. 1.

#### Body weight in Alloxanized mice

The basal body weight (mean) of all groups ranged from (25-30) g and there was no inter-group variation. Administration of alloxan leads to a loss in body weight after three days treatment. On the other hand normal controlled shows increase in body weight<sup>12</sup>. All the data are summarized in the Table 2 and Fig. 2.

#### Histological study

In the histological study, the microscopical observation shows that:

- (i) The pancreatic cells were destroyed with the help of alloxan, which is clear from the slide of the alloxan, induced diabetic mice.
- (ii) The plant extract has regenerative effect on pancreatic tissue, which is clear from the slide prepared from that of the drug treated mice.

Histological study of the pancreatic tissues of control and treated mice shows the scientific justification of folklore use of *C. kyllingia* as an antihyperglycemic agent (Plate 1).

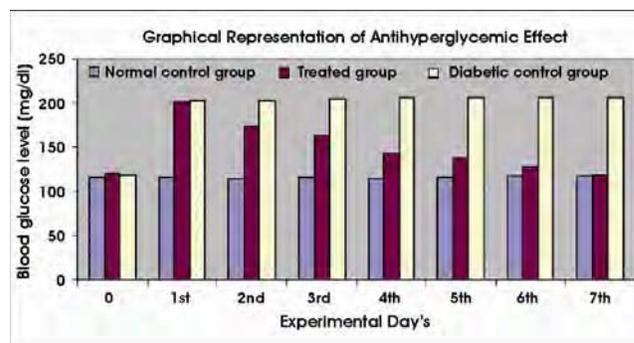


Fig. 1—Effect of feeding dose of *Cyperus kyllingia* on blood glucose levels (mg/dl) in Alloxanized mice

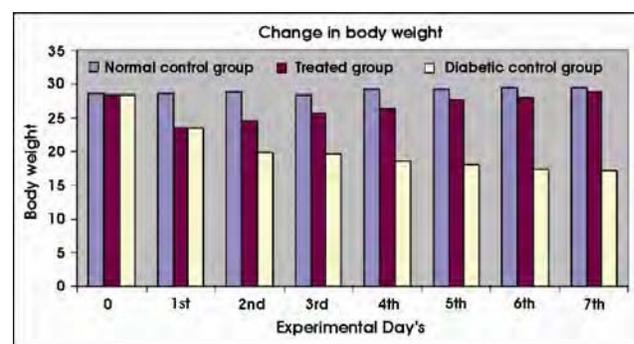


Fig. 2—Effect of feeding dose of *Cyperus kyllingia* on body weight (g) in Alloxanized mice

Table 1—Effect of feeding dose of *Cyperus kyllingia* on blood glucose levels (mg/dl) in alloxanized mice

Groups	0 day	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day	6 <sup>th</sup> day	7 <sup>th</sup> day
NC	116.34 $\pm$ 1.74	116 $\pm$ 1.80	115 $\pm$ 2.38	115.67 $\pm$ 1.36	113.84 $\pm$ 2.05	116.34 $\pm$ 0.95	117.67 $\pm$ 0.72	117.5 $\pm$ 1.57
Treated	120 $\pm$ 0.58	202 $\pm$ 2.03	172.84 $\pm$ 1.49	162.84 $\pm$ 2.62	142.5 $\pm$ 2.06	136.5 $\pm$ 1.18	127 $\pm$ 1.32	118.67 $\pm$ 1.31
DC	118.34 $\pm$ 1.43	203 $\pm$ 1.71	202.5 $\pm$ 1.98	204.34 $\pm$ 2.25	205.5 $\pm$ 2.02	206 $\pm$ 2.11	205.34 $\pm$ 2.08	206 $\pm$ 1.67

Table 2—Effect of feeding dose of *Cyperus kyllingia* on body weight (g) in alloxanized mice

Groups	0 day	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day	6 <sup>th</sup> day	7 <sup>th</sup> day
NC	28.67 $\pm$ 0.99	28.67 $\pm$ 0.84	28.84 $\pm$ 0.75	28.5 $\pm$ 0.76	29.16 $\pm$ 0.65	29.167 $\pm$ 0.65	29.34 $\pm$ 0.56	29.34 $\pm$ 0.56
Treated	28.5 $\pm$ 0.92	23.5 $\pm$ 0.56	24.67 $\pm$ 0.88	25.67 $\pm$ 0.99	26.5 $\pm$ 1.18	27.67 $\pm$ 1.20	28 $\pm$ 1.46	28.834 $\pm$ 1.70
DC	28.34 $\pm$ 0.80	23.34 $\pm$ 0.84	19.834 $\pm$ 0.48	19.67 $\pm$ 0.42	18.67 $\pm$ 0.42	18 $\pm$ 0.58	17.5 $\pm$ 0.43	17.167 $\pm$ 0.60

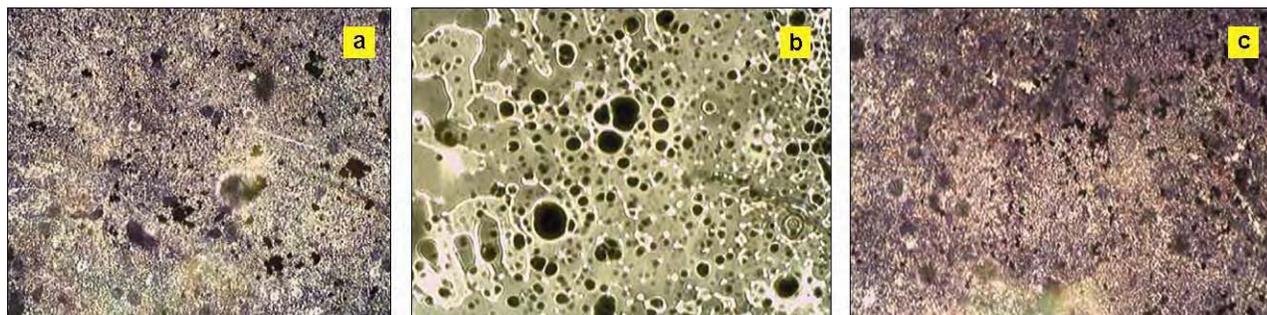


Plate 1—**a**: Histological slide of pancreas of Normal control mice, **b**: Histological slide of pancreas of diabetic control mice, **c**: Histological slide of pancreas of drug (methanolic extract of *Cyperus kyllingia*) treated mice.

#### Statistical analysis

In case of Table 1 and 2 the P-value is less than 0.05 ( $P < 0.05$ ), so the result statistically significant.

#### Conclusion

In present study the efficacy of methanolic extract of *C. kyllingia* as antidiabetic agent in controlling hyperglycemia is investigated. From the aforementioned results, we can conclude that polar part of the extract (water extract) of the plant possesses the capacity to reduce the Fasting Blood Sugar (FBS) and this activity might be due to the reduced insulin secretion in the body.

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