

## Antidiabetic Potentials Of *Cassia occidentalis* Leaf Extract On Alloxan Induced Diabetic Albino Mice

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**Abstract:** *Cassia occidentalis* Linn. is extensively used in folklore medicine to treat several illnesses, even though adequate validation of its hypoglycemic activity has not yet been done. The scientific evaluation of its hypoglycemic activity was, therefore, carried out and compared with the effect of a standard hypoglycemic drug, glibenclamide. In the present study methanolic extract of *C. occidentalis* leaves was tested against alloxan-induced diabetic mice weighing 20-25g. They were randomly grouped into control and experimental. The experimental group mice were induced by a single intraperitoneal injection of alloxan. Treatment with *C. occidentalis* leaf extract at different doses and times following in normal and diabetic mice significantly reduced the blood glucose level to normal in diabetic mice.

**Keywords:** *Cassia occidentalis*, Diabetes mellitus, Glibenclamide, Alloxan monohydrate, Mice.

### Introduction

Diabetes mellitus is a chronic disorder of carbohydrate, lipid, and protein metabolism characterized by persistent elevation of fasting blood glucose above 200 mg/dl, due to insufficient or complete cessation of insulin synthesis or secretion and/or peripheral resistance to insulin action<sup>1</sup>. The disorder is associated with increased risk of heart disease, stroke, kidney disease, retinopathy, neuropathy, ulceration and gangrene of extremities<sup>2</sup>.

Diabetes mellitus is a growing public health concern worldwide causing severe and costly complications<sup>3</sup>. Over 173 million people are suffering from diabetes mellitus in the world and this figure is likely to be increased to 300 million or more by the year 2025<sup>4</sup>. The global incidence of diabetes mellitus for all age groups was estimated to be 2.8% in 2000 and the estimate will reach

4.4% in 2030<sup>5</sup>.

Substantial efforts have been made in recent years to identify antidiabetic agents; a variety of orally active hypoglycaemic agents are frequently used to manage the disease and its other associated disorders. However, the effectiveness of these drugs is limited and prone to a variety of side effects including insulin resistance<sup>6</sup>. These synthetic agents are also relatively expensive for developing countries<sup>7</sup>. Therefore, searching for effective, low cost and less side effect hypoglycaemic agents is important.

Herbal remedies for diabetes are known since ancient times in different societies and available scientific data supports the antidiabetic effects of some medicinal plants<sup>8</sup>. Many traditional plants are used throughout the world to manage diabetes mellitus. A few of the medicinal plant treatments for diabetes have received scientific scrutiny, for which World Health Organization

(WHO) has also recommended attention<sup>9</sup>.

*Cassia occidentalis* belongs to the genus *Cassia* and the Family Caesalpiaceae. It is called Stink weed, Stinking or Negro Coffee. It is an erect herb, commonly found by road sides, in ditches and waste dumping sites. Traditional healers in north central Nigeria have reported that the infusion of the leaves of *C. occidentalis* is used as an effective treatment for hepatitis<sup>10</sup>. The potential of the leaf extract of *C. occidentalis* may be related to its antioxidant activity. The extract contains flavonoids which are powerful antioxidant polyphenolic compounds. The aim of this work is to investigate the antidiabetic potentials of methanolic leaf extract of *Cassia occidentalis* on diabetic mice.

## **Materials and Methods**

### **Experimental Animals**

Male albino mice (25-28 g) procured from the animal farm of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, were used for the experiment. They mice were kept under standard environmental conditions of temperature, relative humidity, dark/light cycle, and were fed with standardized feed pellets and tap water *ad libitum*. The mice were fasted for 12 hours before the experiment but were allowed free access to water. Ethical guidelines in animal handling and use were adhered to strictly in the study.

### **Plant collection and preparation**

Fresh leaves of *Cassia occidentalis* were collected in the month of March, 2011 from Gwagwalada Area Council of the Federal Capital Territory (FCT), Abuja, Nigeria. Gwagwalada is located between latitude 8° 56' 29" North and longitude 7° 5' 31" East within the guinea savanna zone. The area is about 6,350km<sup>2</sup> and is towards the southern part of the FCT and about 500m above sea level. The average annual rainfall is about 950mm and spreads from April to November. The mean ambient temperature range is about 30 to 37°C while relative humidity is 60-70%.

The plant was identified by Prof. B.O. Olorede of Botany Department, University of Abuja, Nigeria. Voucher specimens were deposited in the department's herbarium for reference purposes. The leaves were air dried on laboratory bench top, then pulverized into a coarse powder, sealed in polythene bags and stored at 4°C until used for the study.

### **Extraction of Plant Materials**

A bulk extraction was carried out using about one kilogram of the pulverised leaves of *Cassia occidentalis*. This was done by soaking the plant material in 80% methanol for 48 hours. They were shaken at regular intervals of 2 hours. The extracts were filtered using No. 1 Whatman filter paper and concentrated *in vacuo* to dryness using a rotary evaporator and kept at 4°C. These extracts were used for the preliminary screening.

### **Acute Toxicity Studies**

The acute toxicity of the extract was conducted by the method of Lorke, 1983<sup>11</sup>. Mice fasted for 12 h were randomly divided in to drug treated 'test' groups and vehicle treated 'control' group, total making up six groups of six rats per cage. *Cassia occidentalis* leaf extract (200, 400, 800, 1000 and 1500 mg/kg b.w.) was separately administered orally to the rats in each of the test groups, respectively. Behavioral changes (irritation, restlessness, respiratory distress, abnormal locomotion and catalepsy) were observed over a period of 48 h for sign of acute toxicity. The number of mortality caused by the compound within this period of time was observed.

### **Experimental design**

Hyperglycemia was induced by a single intraperitoneal injection of 150 mg/kg body weight alloxan monohydrate (Sigma Chem. Co., St Louis, MO, USA), freshly dissolved in distilled water immediately before use to overnight feed-fasted albino mice<sup>12</sup>. After 10 days, mice with fasting blood glucose of 6.0mmol/dL or more were considered diabetic and used for the study. Mice were divided into five groups of six mice in each group as follows: group I served as negative control receiving distilled water (10 ml/kg, *per os*); groups II-IV received *Cassia occidentalis* leaf extract at 200 mg/kg, 300 mg/kg, and 450 mg/kg, *per os*, respectively. Group V served as a positive control and received Glibenclamide (2 mg/kg, *per os*). The animals were treated once and fasting blood glucose level measured at 0, 1, 3, 6 and 12 hours. Blood samples were collected by a snip-cut at the tip of the tail under mild anesthesia and blood glucose level was measured using an auto-analyzer - AccuCheck Advantage II glucose kit.

### **Statistics**

All the data are presented as mean ± SEM. The differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by the Dunnett multiple comparisons test.  $P < 0.01$  was considered to be significant.

**Table 1: Effects of *C. occidentalis* on blood glucose levels**

| Blood glucose mmol/L and sampling time |        |            |            |            |            |            |            |
|--|--------|------------|------------|------------|------------|------------|------------|
| Treatment                              | Groups | Weight     | 0hrs       | 1hrs       | 3hrs       | 6hrs       | 12hrs      |
| Negative control<br>(distilled water)  | I      | 26.35±2.25 | 9.20±3.52  | 8.35±2.43  | 7.64±2.14  | 8.38±0.53  | 9.43±4.11  |
| <i>C. occidentalis</i><br>200mg/kg     | II     | 28.63±2.70 | 6.36±4.33  | 4.63±0.75  | 2.23±0.71  | 1.95±0.33  | 1.33±0.93  |
| <i>C. occidentalis</i><br>300mg/kg     | III    | 25.7±2.61  | 8.33±4.73  | 7.57±6.60  | 6.65±6.45  | 4.83±3.79* | 4.35±2.97* |
| <i>C. occidentalis</i><br>450mg/kg     | IV     | 27.78±1.33 | 8.57±3.64  | 6.56±5.34  | 5.94±0.43  | 4.33±2.17* | 3.42±1.12* |
| Positive control<br>(Glibenclamide)    | V      | 25.39±1.42 | 11.17±6.35 | 15.52±9.11 | 7.14±5.17* | 6.38±3.87* | 3.10±0.12* |

\* $P < 0.01$  (Dunnett's multiple comparison test)

## Results

### Acute toxicity

Acute toxicity studies revealed the non-toxic nature of the *Cassia occidentalis* leaf extract. Behavior of the treated mice also appeared normal. There was no mortality or toxic reaction at any of the doses until the end of the study. Table 1 shows the other results of the study.

### Discussion

Diabetic mellitus is a metabolic disease associated with impaired glucose metabolism which in effect alters intermediary metabolism of lipids and proteins adversely. The present study was conducted to study the antihyperglycemic activities of extracts of *C. occidentalis* in mice as well as to provide an introductory approach for the evaluation of its traditional preparation in order to scientifically validate the therapeutic preparation of this plant in the control of diabetes.

Alloxan, a beta cytotoxin, destroys  $\beta$ -cells of islets of Langerhans of pancreas resulting in a decrease in endogenous insulin secretion and paves ways for the decreased utilization of glucose by body tissues<sup>13</sup>. It results in elevation of blood glucose level, decreased protein content, increased levels of cholesterol and triglycerides<sup>14</sup>.

In this study, it was observed that alloxan-induced diabetic mice orally administered methanolic extracts of *C. occidentalis* leaves at the dose of 300 mg/kg produced significant fasting blood glucose lowering activity in 6 and 12 hour samples compared with the control. An increase in the dose of *C. occidentalis* to 450 mg/kg showed more observable hypoglycemic activity in the diabetic mice. This activity is, however, less when compared to that of Glibenclamide.

### Conclusion

Results indicate the presence of active diabetes principles in *C. occidentalis* leaves extract, which seems to confirm its folkloric uses.

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