



## Antihyperglycaemic Efficacy of *Cnidoscopus aconitifolius* compared with Glibenclamide in Alloxan-induced Diabetic Wistar Rats

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### Abstract

Problems associated with the current diabetic pharmacotherapy had further necessitated the need to search for more effective and safer approach to prediabetic and diabetic management. This study investigated the blood glucose lowering efficacy of the chloroform fraction of hydromethanolic leaf extract of *Cnidoscopus aconitifolius* (CA) compared with Glibenclamide in alloxan-induced diabetic Wistar rats. Statistical analysis of the results using SPSS (v. 15) showed that CA caused a decrease in blood glucose level in a dose-dependent fashion. Comparison of means using paired t-test ( $p=0.05$ ) showed that 100 - 200mg/Kg of CA showed significant blood glucose lowering potential. 100, 150 and 200mg/Kg of CA lowered the diabetic blood glucose by 41.76, 71.11 and 73.46%, respectively. 150 and 200mg/Kg CA recorded dose- and time-dependent mortality. The percentage difference in blood glucose level caused by 100mg/Kg CA leaf extract was 39.00% compared with 77.48% caused by 0.5mg/Kg glibenclamide. The blood glucose lowering potential of this novel CA extract could be justified by the strong presence of flavonoids in its phytochemical analysis. Effective dose of CA could prevent the rapid hypoglycaemic side effect in glibenclamide usage. However, the results implied that users of CA should be cautious of its possible dose- and time-dependent toxicity.

**Keywords:** Antihyperglycaemic, *cnidoscolus aconitifolius*, glibenclamide, rats.

### Introduction

Diabetes mellitus is a metabolic disorder found in all nations of the world<sup>1</sup>. It is one of the most prevalent epidemics of the 21st century with significant cause for concern<sup>2-4</sup>. The conventional medical approach of simply using insulin and oral drugs to control diabetes mellitus is inadequate, boring and lack compliance; thus, the patient's exposure to long term complications remains a risk. Some wild herbs and spices have been shown to be most effective; relatively non-toxic and have substantial scientific documentation to attest to their efficacy in diabetes management<sup>5</sup>. Atawodi postulated that traditional medicinal plants have antioxidant properties<sup>6</sup>. He stated that Africa is blessed with enormous biodiversity resources but plagued with several diseases including those with reactive oxygen species (ROS) as their etiological factor. Considerable evidences had been accumulated to implicate cellular damage arising from ROS, at least in part, in the aetiology and pathophysiology of human diseases such as diabetes<sup>7</sup>. Medicinal plant extracts have valuable anti-diabetic effects<sup>8-13</sup>. Due to one or more active components responsible for blood glucose reduction<sup>14,15</sup>. Flavonoids and saponins have been implicated as responsible for the anti-diabetic activity of some plant extracts<sup>16,17</sup>. The antidiabetic mechanism of these phytochemicals is possibly by stimulating insulin release from pancreatic beta cells<sup>18</sup>. Iwuji *et al.* reported recently that

*Cnidoscopus aconitifolius* (*Euphorbiaceae*, CA) hydromethanolic leaf extract obtained from the Niger Delta region contains flavonoids and saponins which possibly may potentiate its antidiabetic activity<sup>19</sup>. The current work is aimed at assessing the antihyperglycaemic / antidiabetic potential of CA, compared with a standard oral antidiabetic drug, glibenclamide (a sulfonylurea).

This study is essential because current therapies seem to be insufficient to prevent diabetic complications in type 2 diabetes, with two to four folds likelihood for developing cardiovascular complications and which necessitates the development of novel health promotion strategies and therapeutic modalities<sup>20</sup>.

### Material and Methods

**Collection of the leaf sample of *Cnidoscopus aconitifolius*:** To obtain potent plant extracts, the age of the plant, the extracting solvent, method of extraction and time of harvesting the leaves were considered<sup>21,22</sup>. Fresh leaf samples of *Cnidoscopus aconitifolius* (CA) collected from private residences at Eleme, Port Harcourt in Nigeria.

Taxonomical Identification of the Plant obtained in Niger Delta, Nigeria.

The plant sample identification was carried out at the Department of Botany and Ecological Studies, Faculty of Science University of Uyo by taxonomists Dr. Mrs. M. E. Bassey and kept as herbarium I Samuel UUH 026113 (Port Harcourt) at the University of Uyo.

**Preparation of crude *Cnidocolus aconitifolius* (CA) leaf extracts:** This was carried out at the Department of Pharmacognosy and Natural Medicine, University of Uyo, Akwa Ibom State, Nigeria.

The fresh leaves of CA were air dried and extraction method was adapted from a previous report<sup>23</sup>. The dried leaves were pulverized with electric grinding machine into minute pieces. Hydro-methanolic (1:4, v/v) extraction was carried out with Soxhlet extractor (Model No. 3567, Austria). The chloroform fraction of the extract was obtained and filtered using Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure in vacuum at 45<sup>o</sup>C using a rotary evaporator (Gallenkamp UK). The resulting residues called dried leaf extracts were transferred to a hot air oven where they were dried to a constant weight at 45<sup>o</sup>C. The extract was stored at 4<sup>o</sup>C.

**Ethical approval for the use of Experimental Animals:** Necessary approval was obtained from the faculty research ethics committee for the use of albino Wistar rats in the study.

**Acquisition and Preparation of Albino Wistar Rats:** The albino Wistar rats were inbred at Jongres Animal house, Owerri Imo State. The rats used were mixture of both sexes and aged between 4-12 weeks weighing between 100 and 200g. The cages and animal house were well ventilated and naturally lightened (12-hourly dark cycle). The rats were randomly grouped in fives into 10 assigned cages for the experimentations. The mean weights were recorded before and after the study. The surrounding of the rats was maintained clean throughout the study period and the rats were fed and allowed access to portable water *ad libitum*. They were fed with pelletized grower livestock feed produced by Vital Feed Limited, Nigeria. (Contents: Crude Protein- 14.50%, Fat-7%, Fibre-7.20%, Calcium- 0.8%, Phosphorus- 0.4%).

**Induction of diabetes:** Prior to induction of diabetes the rats were fasted for 12 hours and the normal fasting blood glucose level was recorded. Then the rats were administered 120mg/kg body weight of alloxan intraperitoneally. The alloxan was produced by Qualikems, India and purchased from a chemical store at Ibadan, Nigeria. The pre-induction and post-induction fasting blood glucose levels were recorded. Diabetes was confirmed by monitoring the fasting blood glucose levels using glucometer and glucose test strips. The blood glucose level of 128-280mg/dl after 24 hours showed that the rats were diabetic. The diabetic and non-diabetic rats were separated and grouped for the study.

**Preparation and Dosing of Drugs used in the study:** Glibenclamide was purchased from Milan pharm. Chemist

LTD, Owerri, Imo State. Two tablets of 10mg were dissolved in 10mls of distilled water forming a solution of 10mg of glibenclamide. A dose of 0.50mg/0.5ml of the solution was orally administered. Normal saline was obtained by dissolving 8.5g of sodium chloride in 1000mls of water. 1.00g of CA was dissolved in 100mls of distilled water.

**Administration of drugs and Monitoring of blood glucose concentrations:** The CA extract and glibenclamide were administered orally using a syringe without needle. Six groups of albino rats (n=5) received 5-200mg/Kg of extract in phase 1 and in phase 2, four groups (n =5) received 50 - 100mg/Kg CA and 0.1 - 0.5mg/Kg of glibenclamide daily for seven days. The pre and post treatment fasting blood glucose levels were obtained and mortality/morbidity cases recorded. Prestige<sup>22</sup> Glucometer with Glucose test strips (code No. 22) was used as specified by the manufacturer. The estimation was made immediately after tail venepuncture with a sterile lancet. Blood glucose level was assayed following a single drop of fresh plasma on the strip properly fixed to the electronic glucose monitor.

**Statistical analysis:** Paired t-tests were carried out with the SPSS (version 15) descriptive statistics. The data were expressed as mean  $\pm$ SD and  $p \leq 0.05$ . Data were also evaluated for effect using the percentage difference.

## Results and Discussion

Groups treated with 5, 10 and 50mg/Kg CA showed no significant reduction of blood glucose level in the diabetic rats and no mortality occurred. Conversely, 100-200mg/Kg CA caused significant reduction of blood glucose level in the diabetic rats. Mortality was not recorded with 100mg/Kg CA and lower doses.

In the next phase, two doses of CA were compared with two doses of Glibenclamide. CA had lesser antihyperglycaemic effect compared with glibenclamide.

Survey in the Niger Delta showed that *Cnidocolus aconitifolius* (CA) is an ornamental plant which are mainly in residential areas, and are used as green vegetables and medicinal plants. The local names (or nicknames) of CA in the Niger Delta of Nigeria are 'ogwu obara' (blood builder) or 'hospital is too far'<sup>19</sup>.

Table 1 shows the mean blood glucose level effect of hydromethanolic extract of CA. All the doses of CA (5-200mg/Kg) were found to reduce blood glucose concentration by 6.25% to 73.46% in the experimental animal model. Although all the doses of CA lowered blood glucose level in alloxan induced diabetic rats the concentrations of 100mg/Kg and 200mg/Kg respectively caused the marked lowering effect. The groups treated with 100mg/Kg had the healthiest significant effect with optimal percentage difference of 41.76%. Statistical

analysis of the results using SPSS (v. 15) showed that CA significantly statistically lowered blood glucose between the mean fasting blood glucose (FBG) concentrations of extract-treated and the untreated diabetic Wistar rats. Comparison of means using paired t-test ( $p=0.05$ ) showed that although the concentration of CA in the order of 100 - 200mg/Kg had significant blood glucose lowering potential, the concentrations in the range 150-200mg/Kg recorded dose- dependent mortality. Thus the numbers of deaths per day were highest with 200mg/Kg followed by 150mg/Kg.

Table 2 shows the blood glucose lowering effect of the extract in comparison with the standard oral antidiabetic drug, glibenclamide. The results depicts that the chloroform fraction of hydromethanolic leaf extract of *Cnidoscopus aconitifolius* has antihyperglycaemic/antidiabetic activity which is 1.99-5.63 times lower than glibenclamide but the safety of both are yet to be compared.

*Cnidoscopus aconitifolius* (CA) leaves have been found to have nutritional qualities and flavonoids that could be antidiabetic<sup>19</sup>.

The consumption of CA leaves obtained in Niger Delta is expected to reduce blood glucose level in pre-diabetic and diabetic patients. It is therefore relevant to study other activities of its antidiabetic effect and identify the active compounds responsible for this effect. CA effective dose of 100mg/Kg is more than 50% potent as 0.1mg/Kg of Glibenclamide. CA could therefore minimize incidence of hypoglycaemic coma associated with most antidiabetic drug misuse.

### Conclusion

The novel chloroform fraction of *Cnidoscopus aconitifolius* hydromethanolic extract had dose-dependent antihyperglycaemic and toxicity potentials. Effective dose of this CA extract could be safer than the fast acting antidiabetic pharmaceuticals like glibenclamide. However, users of CA should be cautious of its possible dose- and time-dependent toxicity. Further study is expected to isolate and identify the antidiabetic compounds present in CA.

**Table-1**  
**The percentage antihyperglycaemic effect of different doses of chloroform fraction of *cnidoscolus aconitifolius* (Ca) in alloxan induced diabetic rats**

Concentration of CA (mg/Kg)	Mean $\pm$ SD FBG of untreated diabetic rats (mg/dl)	Mean $\pm$ SD FBG of treated diabetic rats (mg/dl)	Percent Difference (%)	Paired t-test significance Value ( $p= 0.05$ )
5	197.60 $\pm$ 43.85	185.25 $\pm$ 36.51	-6.25	0.07
10	182.00 $\pm$ 4.84	158.00 $\pm$ 16.84	-13.19	0.47
50	128.60 $\pm$ 10.71	111.60 $\pm$ 13.30	-13.22	0.19
100	182.40 $\pm$ 29.37	106.00 $\pm$ 11.40	-41.76	0.01*
150	280.40 $\pm$ 16.93	81.00 $\pm$ 26.04	-71.11	0.06***
200	233.60 $\pm$ 36.53	62.00 $\pm$ 15.59	-73.46	0.01**

Key:\* = significance difference; \*\*\*=a death/24hrs; \*\*=a death/48hrs; - = antihyperglycaemic

**Table-2**  
**Percentage Antihyperglycaemic Effect Of *Cnidoscopus Aconitifolius* (Ca) And Glibenclamide In Alloxan Induced Diabetic Rats**

S/N	Treatment Group	Mean (diabetic) Control (mg/dl)	Mean Treated (mg/dl)	% difference
1	50mg/kg CA	128.0 $\pm$ 10.60	111.6 $\pm$ 13.29	-12.81
2	100 mg/kg CA	138.0 $\pm$ 19.23	84.2 $\pm$ 23.91	-38.99*
3	0.1mg/kg Glib	146.8 $\pm$ 32.53	41.00 $\pm$ 5.2	-72.07*
4	0.5mg/kg Glib	165.20 $\pm$ 92.06	37.20 $\pm$ 5.89	-77.48*

Key: \* = significance difference; CA = *Cnidoscopus aconitifolius* chloroform extract-fraction; Glib = Glibenclamide - = antihyperglycaemic.

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