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Hypoglycemic Effect of *Cnidoscolus acontifolus* on Alloxan Induced Diabetes in Albino Wistar Rats

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Abstract

The potency of plants is largely due to the presence of phytochemicals contained in it; which establishes its efficacy in the treatment of health conditions like diabetes mellitus. Some obvious limitations of the drug management methods necessitate a search for alternatives among the arsenal of herbs available in the natural habitat of man. Evaluating the hypoglycemic effect of *Cnidoscolus aconitifolus* leaf extract on alloxan induced diabetes mellitus and also to evaluate and compare the hypoglycemic effect of glibenclamide and chloroform, butanol, ethanol extract of *Cnidoscolus aconitifolius* on alloxan induced diabetes mellitus Albino Wistar Rats. Fifthy (50) wistar rats with average weighing 150-200g, were randomly assigned into tengroups of 5animals each. After one week of acclimatization, diabetes was induced with a single intraperitoneal injection of alloxan at a dose of 60mg/kg body weight. Group 1: Served as Negative control (Non-diabetic) and received normal rat chow and 0.3ml normal saline; Group 2: Served as positive control group; groups 3 and 4served as test, and received butanolic and ethanolic extract of *Cnidoscolus aconitifolius* leaf extract respectively orally for 28 days. Groups 5, 6, 7 and 8 received 50mg/kg, 100mh/kg, 150mg/kg, and 200mg/kg respectively while group 9 and 10 received 0.1mg/kg and 0.5mg/kg of glibenclamide respectively. A dose dependent reduction in blood glucose level was observed after treatment when compared with glucose level before induction. This study revealed the ability of *C. aconitifolius* to lower blood glucose level; thereby suggesting that it could serve as a better therapy for diabetes mellitus and paving way for further investigation to identify the actual bioactive compounds responsible.

Keywords: *Cnidoscolus aconitifolius,* glibenclamide, phytocomponents, hypoglycaemic.

1 Introduction

Diabetes mellitus is a disorder that affects the body's ability to make or use insulin. Insulin is a hormone produced in the pancreas that helps transport glucose (blood sugar) from the bloodstream into the cells so they can break it down and use it for fuel. People cannot live without insulin [1]. Diabetes results in abnormal levels of glucose in the bloodstream. This can cause severe short-term and long-term consequences ranging from brain damage to amputations and heart disease [1]. Closely monitoring blood sugar levels in diabetics and checking blood sugar levels in all patients for the development of increased levels is an important nursing function. Control of blood sugar levels can greatly reduce risk and slow development of atherosclerosis [2].

Alloxan, a toxic glucose analogue, has also been used in the induction of diabetes mellitus in experimental animals. It is so called because it inhibits glucose induced insulin secretion in the pancreatic B cells [3] and selectively destroys the B cells as it accumulates in these cells via Glut 2 glucose transporters [4]. Within the cell thereafter, it generates hydroxyl radicals from its reaction with the intracellular thiols (glutathione) to form dialuric acid. Subsequent auto oxidation of dialuric acid generates superoxide radicals, hydrogen peroxide as well as hydroxyl radicals. The hydroxyl radical so formed is then responsible for destruction of the B cells and the ensuing insulin dependent diabetes. The prevalence of diabetes mellitus in some countries has reached 1-2% of the total population and in Africa, especially in Nigeria, it is on the increase [5].

The term diabetes mellitus describes a metabolic disorder of multiple etiologies and is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism defects due to absolute or relative lack of insulin produced in the pan creases which aid the transport of glucose from the blood stream to the cells [6].

There are basically two main classes of diabetes mellitus: Type 1 (Insulin-dependent diabetes mellitus, IDDM). Diet and exercise are crucial in managing diabetes, especially type 2 diabetes (NIDDM) and gestational diabetes. Careful control is needed to reduce the risk of long-term complications. This is theoretically achievable to combina-

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tions of diet, exercise and loss (type2) various oral diabetic drugs (type2 only), and insulin use(type 1 and for type 2 not responding to oral medications, mostly those with extended duration diabetes) in addition, given the associated higher risks of cardiovascular disease, lifestyle modifications should be undertaken to control blood pressure and cholesterol by exercising more, smoking less or ideally not at all, consuming an appropriate diet, wearing diabetic socks, wearing diabetic shoes, and if necessary, taking any of the several drugs to reduce blood pressure[7]. Some obvious limitations of the drug management methods necessitate a search for alternatives among the arsenal of herbs available in the natural habitat of man [7].

In these study, we aim at evaluating the hypoglycaemic effect of chloroform extract of *Cnidoscolus aconitifolus* leave on alloxan induced diabetes mellitus and also to evaluate and compare the hypoglycaemic effect of glibenclamide and chloroform extract of *Cnidoscolus aconitifolius* on alloxan induced diabetes mellitus Albino Wistar Rats.

2 Experimental

Collection and identification of plant material

Fresh leaves of *Cnidoscolusaconitifolius*(CA) were gotten from a private residence in sabongari, kano, kano state Nigeria in 2019. Identification and taxonomical classification were carried out at Department of Botany, Bayero University kano, Nigeria.

Preparation of *Cnidoscolusaconitifolius* (CA) leaf extracts.

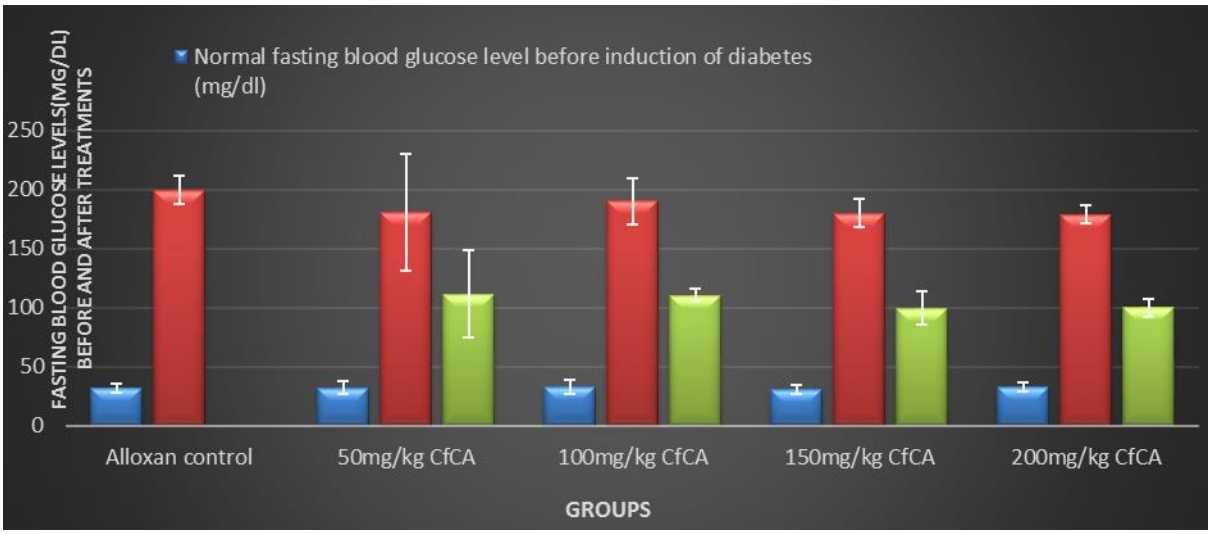
The fresh leaves of CA were air dried. The dried leaves were pulverized with electric grinding machine into minute pieces. Hydro-methanolic (1:4, w/v) was carried with soxhlet extractor. At the end of the extraction, the extract was filtered using whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure in vacuum at 45^{o}C using a rotatory evaporator.

Experimental design

The animals were administered different doses of the extract for 28 days based on the varying individual body weight. Illustrated further as;

Group 1- given 0.3ml normal saline (negative control).
Group 2- intraperitoneally induced alloxan (diabetic control).
Group 3- diabetic rats treated with 50 mg/kg CA butanol fraction.
Group 4- diabetic rats treated with 50 mg/kg CA ethanol fraction.
Group 5- diabetic rats treated with 50 mg/kg CA chloroform fraction.
Group 6- diabetic rats treated with 100 mg/kg CA chloroform fraction.
Group 7- diabetic rats treated with 150 mg/kg CA chloroform fraction.
Group 8- diabetic rats treated with 200 mg/kg CA chloroform fraction.
Group 9- diabetic rats treated with 0.1 mg/kg Glibenclamide.
Group 10- diabetic rats treated with 0.5mg/kg Glibenclamide

Fifty (50) rats of both sexes weighing 100-250g was used with Five (5) rats in each group. The animals used for this study are albino rats obtained from the animal house at Bayero University Kano. They were fed with vital feed. During the experiments, the rats were kept in twelve (12) groups (of 4 or 5 animals per group) at ambient temperature on 12hours light, 12 hours' dark cycle. These rats were fed normal rat chow and water. Acclimatization was allowed for two weeks before Alloxan was administered and treatment was done for four weeks.



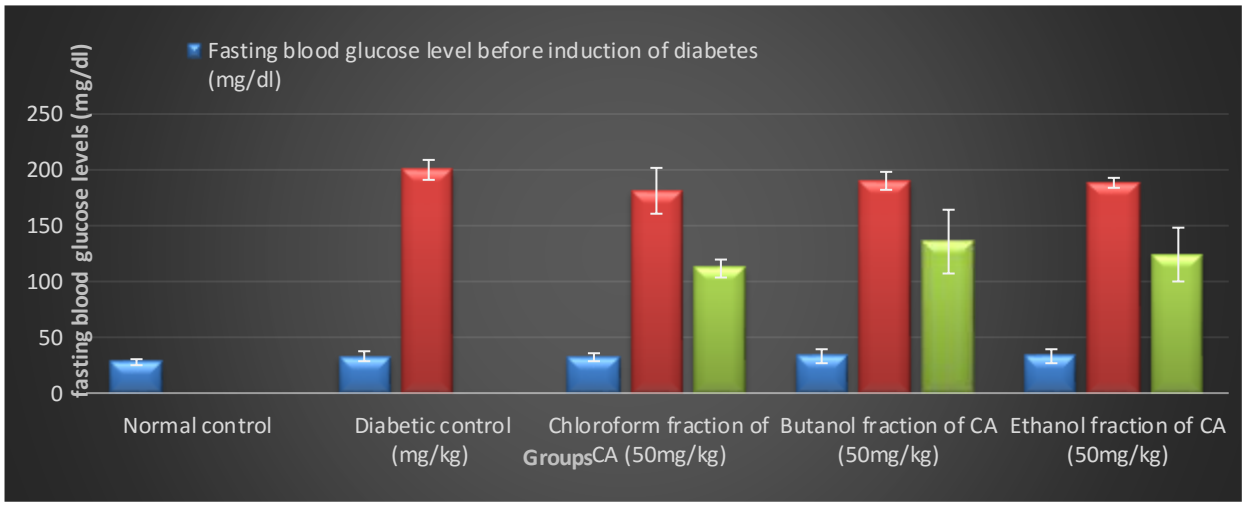
**significance increase at (p<0.05)) between experimental group and control for diabetic induced albino wistar rats and a significance decrease at (p<0.05) between control group and experimental animals for post treated fasting blood glucose level*.

Figure 1. Effects of *Cnidoscolus aconitifolius* leaf extract on fasting blood glucose levels on diabetic induced albino wistar rats.

The cages were cleaned daily to prevent infection of animals and animals care and treatment were conducted in conformity with the institutional guidelines which are in compliance with guide line for the care of laboratory animals, United States National Research council, 1996. After administration of 120mg/kg of alloxan, the fasting blood glucose levels in the experimental groups rose in the experimental groups confirming a diabetic state with statistical significance increase at ($p<0.05$) between control group and groups 5,6,7 and 8.

There was also a siginificance decrease at ($p<0.05$) between control groups and experimental groups of post treatment fasting blood glucose levels of groups 5,6,7 and 8 indicating a significance decrease in the diabetic fasting blood glucose level. There was a significance increase at ($p<0.05$) between control group and experimental groups for post treated fasting blood glucose level for group 5 and 6 indicating a less hypoglycaemic state.

Thus, the extract can be said to be toxic even though not confirmed at the level of group 7 and group 8 with an interval death time of 24hours and 48hours respectively due to the death rate. 0ne (1) death was recorded in group 6 at an interval of 72hours. No death was recorded in other groups indicating group 6 to be the most effective dose as it showed even a more hypoglycaemic effect. Increased death rate was observed in alloxan induced diabetes before treatment at an interval of 24hours.This indicates that diabetic patients have an increased incidence and higher probability of mortality rate when they remain without treatment.



**Significance decrease at ($p<0.05$) of the experimental groups compared to the control.*

Figure 2. The hypoglycaemic effect of different fractions of *Cnidoscolus aconotifolius* on diabetic induced albino wistar rats.

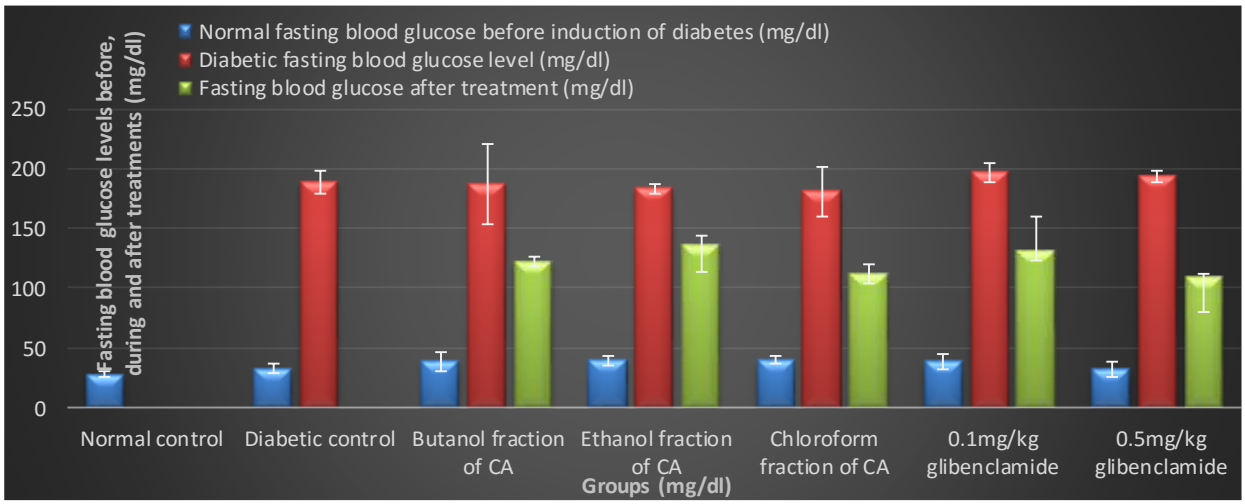


Figure 3. The hypoglycaemic effect of fractions of *Cnidoscolus aconitifolius(CA*) and Glibenclamide (a standard anti diabetic drug) on diabetic induced albino wistar rats

Chloroform fraction of *Cnidoscolus aconitifolius* leaf extract showed a higher statistical significance decrease at (p< 0.05) indicating a higher hypoglycaemic activity compared to the control and the pre-induced diabetic glucose level. Butanol fraction showed a significance decrease at (p<0.05) for fasting blood glucose level of post-treated diabetic rats but, lesser when compared to group chloroform and ethanol fraction. Thus, other concentrations of chloroform extract of CA was pursued for further study in this article.

Group 9 indicates a lower dose of glibenclamide and show a less significance decrease at (p<0.05) compared to group 10 thus, indicates that glibenclamide is dose dependent. Group 5 showed a higher significance decrease compared to group 3, 4, 9 and 10 confirming a more hypoglycaemic effect.

Table 1. Effect of different doses of chloroform fraction of *cnidoscolus aconitifolius (CA)* leaf extracts on the fasting blood glucose levels

S/N	Groups	Mean±SD Fasting blood glucose level before induction of diabetes (mg/dl)	Mean ±SD Diabetic induced fasting blood glucose level (mg/dl)	Mean ±SD Post treatment fasting blood glucose level (mg/dl)	% change
1.	Normal Control	27.5 ±2.35	0.00	0.00	0.00
2.	Diabetic control	32.20 ±3.77	188.60 ±12.33	0.00	0.00
3.	0.1mg/kg of Glibenclamide	35.00 ±5.19	197.60 ±9.02	131.60 ±8.51	33.40
4.	0.5mg/kg of Glibenclamide	39.40 ±6.18	194.00 ±8.39	108.00 ±5.44	44.30
5.	50mg/kg CfCA	32.2 ±3.74	180.60 ±12.33	111.60 ±854*	36.44
6.	100mg/kg CfCA	33.0 ±3.86	190.00 ±7.79	110.20 ±7.77	42.00
7.	150mg/kg CfCA	31.00 ±5.72	180.00 ±12.00	99.60 ±10.34	44.67
8.	150mg/kg CfCA	32.40 ±5.72	179.00 ±10.39	99.70 ±9.78	44.30

**significance increase at ($p \leq 0.05$) between experimental group and control for diabetic induced albino wistar rats and a significance decrease at ($p \leq 0.05$) between control group and experimental animals for post treated fasting blood glucose level.*

Table 2. Shows the fasting blood glucose levels after treatments and weights of rats before and after treatments.

S/N	Groups	Mean ± SD Weight before treatment (g)	Mean±SD Weights of rats after treatment(g)	% change
1.	Normal Control	62.60 ±10.34	80 ±7.53	27.7
2.	Diabetic Control	136.20 ±3.35	120 ±13.36	-11.91
3.	0.1mg/kg glibenclamide	153.54 ±12.19	139.70 ±29.11	9.0
4.	0.5mg/kg glibenclamide	222.98 ±8.55	169.18 ±10.71	69.97
5.	50mg/kg Chloroform fraction of CA	156.20 ±12.96	112.80 ±4.87	27.78
6.	50mg/kg Butanol fraction	96.10 ±3.13	89.20 ±24.00	7.18
7.	50mg/kg Ethanol fraction	156.10 ± 3.15	133.02 ±27.92	14.67

There was a significance increase at (p<0.05) between control group and experimental groups for post treated fasting blood glucose level for group 3 and 4 indicating a less hypoglycemic.

As shown in table 1 above, there was a great reduction of glucose level after four weeks of treatment. From the table, we observed a percentage change in glucose level was dosage dependent. Also, we observed that chloroform extract is most effective at 150mg/kg.

There was a statistical increase at (p<0.05) in weights of rats in group 1 given 0.03ml of normal saline when compared to the initial value. The other groups (2, 3, 4, 5 and 7) showed a significance decrease at (p<0.05) in the weights after treatment with the exception of group 6

which showed a slight significance decrease at ($p<0.05$) in the weight after treatment

Naturally, diabetic rats tend to lose weight due to so many factors which include lose of water via frequent urination, metabolism etc. in table 2, it was clearly observed that after treatment, diabetic rats began to gain weight as the level of glucose in the blood began to reduce. Group 8 & 10 (those treated with glibenclamide which is a standard diabetic drug) gained weight even though it was found to be dosage dependent.

Table 2 also showed that chloroform extract of CA was more effective than the butanolic and methanolic extract. Although they butanolic and ethanolic extract showed slight increase in weight but not as much as the chloroform fraction.

The study investigated the hypoglycaemic effects of the ethanolic, butanol and chloroform extracts of *Cnidoscolus aconitifolius* extracts on diabetic induced albino wistar rat.

From the above results, figure 2 showed the most effective doses at which chloroform fraction of *Cnidoscolus aconitifolius (cfCA)* leaf extract can be administered. The extract showed a higher significance decrease at ($p<0.05$) of the fasting blood glucose level after treatments when compared to diabetic control, pre-blood glucose level and fasting blood glucose level after induction of diabetes at 50mg/kg and 100mg/kg thus, confirms the most effective dose of the extract. Furthermore, no death was recorded throughout the period of experiment in group given 100mg/kg of the chloroform fraction of extract but, two death were recorded at interval of 24hours after administration of extract in the group given 200mg/kg thus confirms that the extract maybe toxic at this dose although not confirmed. However, there was a significance decrease at ($p<0.05$) in the fasting blood glucose level after treatment but less hypoglycaemic effects were observed when compared to the group given 100mg/kg and 150mg/kg. One death was recorded in the group given 150mg/kg of CfCA at interval of 24hours. However, the group showed a significance decrease in the fasting blood glucose level after treatment but less when compared to the group given 100mg/kg and show more hypoglycaemic when compared to the group given 200mg/kg.

Figure 2 showed the hypoglycaemic effects of different fraction of *Cnidoscolus aconitifolius* on diabetic induced rats. Groups given 50mg/kg of chloroform fraction of *Cnidoscolus aconitifolius* showed a more significance decrease at ($p<0.05$) of the fasting blood glucose levels after treatment when compared to the groups given 50mg/kg each of butanolic and ethanolic fractions. Group given butanol showed a less significance decrease in glucose level compared to chloroform and ethanol fractions of CA and also showed a very less significance decrease in body weight when compared with other fractions of CA. There was a high close markedly significance decrease in weights of rats between chloroform and ethanol fraction when compared to ethanol fraction. Glibenclamide (a standard antidiabetic drug) was used as the positive control and showed a more hypoglycaemic at a high dose of 0.5mg/kg when compared to the group given 0.1mg/kg. Chloroform fraction of CA showed a more hypoglycaemic when compared with other fractions of CA to 0.5mg/kg of glibenclamide[12].

Identified flavonoid as one of the bioactive compounds present in *Nauclea Latifolia fruit.* Flavonoid has been associated with antioxidant and free radical scavenging activities [13]. Alloxan causes a massive reduction in insulin release by the destruction of b -cells of the islets of Langerhans and thereby induces hyperglycemia [14]. Daily administration of aqueous *Cnidoscolus aconitifolius* (CA) for 28 days hadresulted in a decrease in blood glucose level in alloxan-induced diabetic rats. Phytochemical screening who showed that flavonoids are present. Flavonoids are very good anti-oxidants that fight against free radicals [16].

4 Conclusions

From the afore mentioned investigations, it can be seen that chloroform fraction of *Cnidoscolus aconitifolius* (CA) has a more hypoglycaemic effects when compared to fractions of butanol and ethanol.

Conflict of Interest

No conflict of interest among researchers.

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