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Antidiabetic Potential of the Aqueous Leaf Extract of *Cnidoscolus aconitifolius* on Streptozotocin (STZ) Induced Diabetes in Wistar Rat Hepatocytes

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Abstract: *Cnidoscolus aconitifolius* has been reported to exhibit hypoglycaemic property and hence recommended traditionally for the treatment of diabetes mellitus. However, the effect of *Cnidoscolus aconitifolius* leaf extract on the biochemical complication of Streptozotocin (STZ) induced-diabetes is yet to be scientifically verified. Body weight changes, blood glucose and serum lipids were assessed as indicators of diabetes severity and complication in this present study. 60 mg/kg body weight of STZ was administered to male Wistar rats intraperitoneally once as a single dose. In a dose dependent manner (100 mg/kg and 200 mg/kg), the aqueous leaf extract were administered orally (by intubation) as single daily dose for a routine period of 21 days. Relative to the control, STZ treatment significantly increased ($p<0.05$) blood glucose from 90.61±5.9 mg/dL (Control) to 237.70±18.7 mg/dL (STZ group alone). Results further indicated that *Cnidoscolus aconitifolius* treated group significantly ($p<0.05$) decreased blood glucose level in a dose dependent manner when compared with STZ induced diabetic group. Coupled with the loss in body weight and disturbed lipid homeostasis (serum total-cholesterol, LDL cholesterol, and TAG) in the diabetic group, *Cnidoscolus aconitifolius* significantly ($p<0.05$) returned the changes in body weight and lipid profile close to control values. Serum lipids were significantly ($p<0.05$) decreased except for serum HDL-cholesterol that was increased by the extract when compared with the STZ treated group. The findings obtained from this study suggest that in STZ- induced diabetic rats, aqueous leaf extract of *Cnidoscolus aconitifolius* may be effective for the treatment of insulin dependent diabetes mellitus.

Key words: Blood glucose, body weight, *Cnidoscolus aconitifolius*, lipid profile, streptozotocin (STZ)

INTRODUCTION

Cnidoscolus aconitifolius commonly known as Chaya and "Iyana Ipaja" in Yoruba language belongs to the family of Euphorbiaceae. It is an evergreen; drought deciduous shrub up to 6m in height with alternate pinnate lobed leaves, milky sap and small flowers on dichotomously branched cymes (Awoyinka *et al*., 2007).The part of the plant mainly used for medicinal purposes is the leaf and shoot. A wide variety of claims have been made for its medicinal efficacy as a treatment for numerous ailments ranging from improved digestion, stimulating lactation and strengthening of fingernails (Rowe, 1994), to cure alcoholism, insomnias, gout and vision improvement (Jensen, 1997; Atuahene *et al*., 1999). Recently, it has also been recommended traditionally in the treatment of diabetes (on which this research is based), obesity, kidney stones, hemorrhoids and eye problems (Diaz-Bolio, 1975; Kuti and Torres, 1996). The plant has been analyzed to contain high amounts of protein, crude fiber, calcium, vitamin C and carotene (Kuti and Torres, 1996). Although this plant has been associated and implicated in the treatment of diabetes, its mechanism of action still remains obscure. There are little or no scientific evidences to substantiate its efficacies and effectiveness in the treatment of this disease.

Diabetes is a disease associated with either a shortage of or resistance to insulin. Insulin is needed to help the body process blood sugar, converting it to energy and proper cell function. Reports have shown that diabetics are at high risk of developing atherosclerosis through a variety of mechanisms (Beckman *et al*., 2002). Atherosclerosis which is often called the "hardening of the arteries" is caused by the reduced arterial blood flow to vital organs. High concentration of blood glucose can irritate the lining of the arteries that promotes the accumulation of "plaque" (Shah *et al*., 2010). More so, the abnormal level of circulating glucose can lead to high concentration of "free radicals" (Hayashi *et al*., 2008). Nowadays, the frequency of diabetes mellitus is increasing many folds. Researches have shown that it is the body composition mainly body lipids that are responsible for increased prevalence of this disease (Arora *et al*., 2007). Hence the purpose of this present study is to evaluate the possible antidiabetic potential of the aqueous

leaf extract of *Cnidoscolus aconitifolius* by investigating its hypolipidaemic properties in Wistar rats.

MATERIALS AND METHODS

Plant material: Fresh leaf samples of *Cnidoscolus aconitifolius* were collected form an uncultivated farmland at the University of Benin, Edo state, Nigeria. Botanical identification was carried out at the herbarium (FHI) Forestry Research Institute of Nigeria, Ibadan, Oyo State. The voucher number obtained was FHI.108788.
Preparation of the aqueous plant extract:

The method of leaf process was carried out according to Awoyinka *et al*. (2007). Fresh leaves of *C. aconitifolius* was air-dried and ground into uniform powder using Thomas contact Mill (Pyeunicam, Cambridge, England). A quantity of the ground sample was soxhlet extracted using 500 mL distilled water for 12 h at 100ºC. The extract was evaporated to dryness using grant instrument (Cambridge, England) at 45º-50ºC to a yield of 20 g plant material which was dissolved with appropriate volume of distilled water to the desired concentration.

Experimental animals: Twenty four male Wister rats (180-300 g) used for this study were purchased from the Animal Unit, College of Medicine, Ambrose Ali University, Ekpoma, Edo State Nigeria. They were divided into four experimental groups of six rats per group. Members of each group were housed in a standard rat cage (Griffin and George Modular Cage System, Model YSM 580 cage base and YSM 600-540 cage top) and allowed to acclimatize to laboratory condition for one week. All rats were allowed free access to drinking water and rat feed (chow) - product of Edo Feeds and Flour Mill (EFFM), Ewu, Edo State, Nigeria.

Induction of diabetes in rats: After one week of acclimatization, diabetes was induced with a single intraperitoneal injection of Streptozotocin (STZ) at a dose of 60mg/kg body weight, after 18 hours fast according to the method described by Bonner-Weir *et al*. (1981). The STZ was freshly dissolved in citrate buffer (0.01M, pH 4.5) (Ozsoy-Sacan, 2000).The injection volume was prepared to contain 1.0 mL/kg (Murali *et al*., 2002). After 5days, blood glucose levels were measure and the animals with a concentration of more than 230mg/dL were classified as diabetic (Cetto *et al*., 2000).

Experimental design: The rats were randomized into four groups of six animals each:

Group I: (Control), Received normal saline solution (0.9% NaCl w/v, 5 mL/kg)
Group II: Received STZ alone (60 mg/kg body weight) once
Group III: Received STZ (60 mg/kg body weight) once before receiving the aqueous extract of *Cnidoscolus aconitifolius* (100 mg/kg body weight) 30 min after STZ administration
Group IV: This group was administered STZ (60 mg/kg body weight) once with 200 mg/kg body weight of *Cnidoscolus aconitifolius*. Administration was carried at the same time and in the same manner as in Group III

Measurement of blood glucose levels: The body weight and blood glucose level were measured at the beginning and end of the experiment. Blood samples were obtained by tail vein puncture of both the normal and STZ-induced diabetic rats. Blood glucose bevels were determined using a glucometer (Lifescan Johnson and Johnson Company, Milipitas, CA).

Preparation of serum homogenate: The animals were sacrificed 21days after treatment. The blood was collected by heart puncture into tubes without anticoagulant to separate serum for various biochemical estimations. The serum was separated by centrifugation at 3000 rpm for 10 min. The obtained supernatants were stored at 4ºC until required for assay

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Biochemical assay: Serum Triacylglycerol (TAG) was quantified by enzymatic end point colorimetric method (Searcy, 1969), while the HDL- cholesterol fraction was measured by the enzymatic-colorimetric method (Burstein and Mortin, 1969). Serum total-cholesterol was estimated by enzymatic colorimetric method as described by Allain *et al*. (1974) and serum LDL-cholesterol was estimated mathematically (Friedewald *et al*., 1972). The reagents for the assays were already prepared and packaged in commercial kits supplied by Randox Laboratories, United Kingdom.

Statistical analysis: The data were presented as Mean ± SD. The mean values of the various treatment groups were compared using ANOVA (Lapin, 1978). The significant level was set at $p<0.05$.

RESULTS

The results of blood glucose level and body weight change in control, STZ-induced diabetic rats and *C. aconitifolius* treated diabetic rats were show in Table 1. There was a significant ($p<0.05$) increase in blood glucose levels in STZ-induced diabetic rats (Group II) when compared with control rats (Group 1). Administration of aqueous leaf extract of *C. aconitifolius* at a dose of 100 and 200 mg/kg body weight significantly ($p<0.05$) decreased blood glucose in STZ induced rats (Group III and IV). The result was dose dependent. Body weight changes of the diabetic group (Group 2) was significantly decreased ($p<0.05$) when compared with the control group. The diabetic rats treated with the aqueous extract, had body weights returned to near reference

Table 1: Effect of aqueous leaf extract of *Cnidoscolus aconitifolius* on experimental and control rats

	Body weight (g)			Fasting blood glucose (FBG) (mg/dL)	
Treatment	Initial	Final	Change	Initial	Final
Group I (control)	259.77±9.0	278.58±6.7	18.81±4.8**	93.46±6.8	90.61±5.9
Group II (STZ) alone	263.42±7.1	260.46±4.4	-3.96±2.7	217.35±6.2	237.70±10.7
Group III (STZ + 100 mg/kg extract)	265.70±6.9	275.20±6.3	9.50±4.3*	212.81±7.9	171.43±0.60^{y}
Group IV (STZ + 200 mg/kg extract)	270.55±9.4	286.44±7.5	15.89±3.6**	229.20±10.5	128.66±7.7

^{y}

Values were expressed as Mean±SD of 6 rats each per group; *: Significantly different from the STZ-induced diabetic Group II ($p<0.05$); **: Significantly different from the STZ-induced diabetic Group II and Group III ($p<0.05$); ^{y}: Significantly different from initial FBG ($p<0.05$)

Table 2: Effect of the aqueous leaf extract of *Cnidoscolus aconitifolius* on serum lipids induced by STZ and control rats

Treatment	Total-cholesterol (mg/dL)	HDL-cholesterol (mg/dL)	LDL-cholesterol (mg/dL)	TAG (mg/dL)	VLDL-cholesterol (mg/dL)
Group I (Control)	107.3±5.8**	53.6±5.1*	45.8±3.3**	63.7±3.1**	10.8±2.7*
Group II (STZ) alone	188.5±7.7 (76%)^{x}	28.9±4.7 (46%)^{x}	122.2±3.6 (167%)^{x}	147.6±5.3 (132%)^{x}	37.5±4.1 (247%)^{x}
Group III (STZ +100mg/kg extract)	143.8±8.1* (34%)^{x}(24%)^{y}	41.2±5.0* (23%)^{x}(43%)^{y}	81.5±2.4* (78%)^{x}(33%)^{y}	114.3±5.7* (79%)^{x}(23%)^{y}	22.9±6.4* (112%)^{x}(39%)^{y}
GroupIV (STZ +200mg/kg extract)	111.4±5.5** (4%)^{x}(41%)^{y}	44.9±7.2* (16%)^{x}(55%)^{y}	55.2±2.7** (21%)^{x}(54%)^{y}	69.4±2.5** (9%)^{x}(53%)^{y}	13.9±3.0** (29%)^{x}(63%)^{y}

Values were expressed as Mean±SD of 6 rats each per group; *: Significantly different from the STZ-induced diabetic Group II ($p<0.05$); **: Significantly different from the STZ-induced diabetic Group II and Group III ($p<0.05$); ^{x}: % Change with respect to Control Group I; ^{y}: % Change with respect to the STZ treated p Group II

range. Changes in the levels of serum lipids in control and experimental rats were illustrated in Table 2. The total cholesterol, LDL - cholesterol and triacylglycerol were significantly increased, while HDL-cholesterol indicated a significantly decreased value in STZ-induced diabetic rats (group II) ($p<0.05$) when compared with the control (Group I) rats. The aqueous leaf extract of *Cnidoscolus aconitifolius* (100 mg/kg and 200 mg/kg body weight) offered a significant protection against alteration in the serum lipids of diabetic rats. The results were also dose dependent.

DISCUSSION

The aqueous hypolipidaemic potential of leaf extract of *Cnidoscolus aconitifolius* was evaluated. Both serum glucose and lipid profiling have been shown to be important predictors for metabolic disturbances including dyslipidaemia, hypertension, diabetes, cardiovascular diseases, hyperinsulinaemia e.t.c. (Slyper, 1994). Alterations in the levels of lipids and glucose in the body increase the risk of lipid associated diseases.

Streptozotocin (STZ) caused a significant elevation in the level of blood glucose in rats (Table1). The administration of 100 and 200mg/kg body weight of the aqueous leaf extract of *Cnidoscolus aconitifolius* significantly reduced blood glucose level, suggesting that it has a hypoglycaemic property. Furthermore, the change in body weight of the rats given the plant extract showed a significant effect in improving body weight loss in a dose dependent manner when compared with the STZ group alone (Group II).

Recently, research findings have shown that increased in body components, mainly body fats and lipid profiles are responsible for increased prevalence of diabetes (Arora *et al*., 2007). The data obtained from this study appears to support this initial observation (Table 2). Beckman *et al*. (2002) observed that complications of atherosclerosis cause most morbidity and mortality in patients with diabetes mellitus. Atherosclerosis which causes reduced arterial blood flow to vital organs has been associated with increased TAG, total-cholesterol, LDL-cholesterol and decreased HDL-cholesterol levels (Mahley *et al*., 1991). From Table 2, the group that received STZ only indicated an increase in serum VLDL and a decrease in HDL-cholesterol when compared with other groups.

An increase in VLDL might have occurred in diabetic mellitus rats due to increase availability of glucose for VLDL synthesis and decrease in lipoprotein lipase activity leading to decrease in VLDL in peripheral circulation (Arora *et al*., 2007). Groups that received the plant extracts (III and IV) showed a reduced level of VLDL. Observation from Table 2, showed that the groups that received the plant extract had reduced LDL-cholesterol and increased HDL- cholesterol, respectively when compared with the diabetic group without the extract. The possible reason(s) for this is obscure, but it coincided with previous study which showed increase HDL-cholesterol and decreased LDL-cholesterol decreased risk factors for metabolic syndrome (Wirth and Steinmetz, 1998).

Since hpyerlipidaemia and hyperglycaemia are risk factors for the prevalence of this disease, *Cnidoscolus*

aconitifolius extract should be further purified and characterized for possible application as an antidiabetic agent.

CONCLUSION

Nowadays frequency of diabetes mellitus is increasing many folds. Research findings show that it is the body composition components, mainly body fat and lipid profiles that are responsible for increase prevalence of this disease. This study verifies the fact that consumption of aqueous extract of leaves of *Cnidoscolus aconitifolius* has hypoglycaemic effect. Results obtain showed that aqueous extract of leaves of *Cnidoscolus aconitifolius* could be speculated as an effective adjunct in the management of diabetes mellitus, but the mechanism by which it decreases glucose level should be subsequently investigated and the principle characterized.

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