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EFFECT OF *Cnidoscolus Aconitifolius* LEAF EXTRACT ON THE BLOOD GLUCOSE AND INSULIN LEVELS OF INBRED TYPE 2 DIABETIC MICE

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Abstract – The effects of *Cnidoscolus aconitifolius* (CA) leaf extract and chlorpropamide on blood glucose and insulin levels in the inbred type 2 diabetic mice are reported. After treatment with CA, the glucose levels were measured at 0 and 2-hour intervals in experimental groups and controls. Group I received no treatment and served as control; Group II was the reference and it received chlorpropamide; Groups I-III were moderately diabetic, 100-300 mg/dL blood glucose levels while Group IV were severely diabetic (> 300 mg/dL). Groups III and IV received CA and served as test groups. There was no significant difference between the blood glucose levels at 0 and 2 hours for the control group, ($P>0.23$) but there were statistically significant differences for Group II ($P<0.0002$); Group III ($P<0.002$) and Group IV ($P<0.0001$). For moderately diabetic mice, CA and chlorpropamide decreased the glucose levels by 25.6% and 16.3% respectively while for the severely diabetic mice CA decreased the blood glucose by 43.7%. It is proposed that CA has an insulinogenic property that possibly stimulated dormant β-cells to secrete insulin. The histopathology of several organs in the treated animals was found to differ from the expected. The islets of Langerhans for example were found to be preserved in the time frame examined. Also the liver and kidney were found to display milder pathology in the treated groups.

Key words: *Cnidoscolus aconitifolius*, plant extract, Type II Diabetes, blood glucose, plasma insulin, inbred diabetic mice, chlorpropamide.

INTRODUCTION

The synergistic advantages of polypharmacy in herbal medicine have shown important growth and development. Research in this area has produced examples of medically relevant results and therefore continues to offer potential. An example of such alternative medicine, as related to diabetes treatment, is based on a plant, *Cnidoscolus aconitifolius* (P. Mill and I.M. Johnston), family *Euphorbiaceae*, also known as cabbage-star, and tree-spinach (40). This plant yields products that are used for diabetes management by some traditional healers in southwestern Nigeria. Their approach is to make a powder from the edible leafy part of C. *aconitifolius*. From this powder an aqueous or ethanolic solution is produced and administered orally. Some people drink the raw liquid after overnight soaking or boiling the dried plant materials (Oladeinde, Personal Communication, 2003). Follow-up in terms of insulin or glucose levels are not known. In Nigeria, over 3 million have been diagnosed with diabetes (36). So there is great potential for the spread of this traditional treatment.

C. aconitifolius is the only plant that has economic food value out of the six *Cnidoscolus* species. The other species, namely: *C. chayamansa, C. elasticus, C. stimulosus, C. texanus, and C. urens* are poisonous except *C. elasticus* used for manufacturing latex/rubber (3,

Abbreviations: **CA**, *Cnidoscolus aconitifolius ;* **DM**, Diabetes Mellitus ; **IACUC**, Institutional Animal Care and Use Committee; **NCCAM**, National Center for Complementary and Alternative Medicine*;* **ADA**, American Diabetes Association*;* **NIH**, National Institute of Health; **RCMI**, Research Center in Minority Institution*;* **CAM**, Complementary and Alternative Medicine; **MSU**, Morgan State University; **IRB**, Institutional Review Board

40). CA is also found in southern Mexico and Costa Rica, and has been introduced into the USA in southern Texas and Florida as a leafy vegetable and/or as a medicinal plant by the Hispanics (6, 30). The plant is known as 'chaya' in south Texas and is prepared as tea by the Hispanics to treat symptoms of non-insulin dependent type II diabetic disease (18). The cooking of the leaves of CA is essential prior to consumption so as to remove the toxic hydrocyagenic glycosides (17). In our preliminary investigation, no toxic cyanogenic glycosides were detected in our laboratory using both traditional overnight soaking and conventional boiling method (27). The cooked leafy part is also good dietary sources of vitamins (ascorbic acid, riboflavin, thiamine, and beta-carotene), minerals (Ca, Mg, Na, K and Fe), protein and fibers (17). The nutritional importance of the leaf fiber has also been demonstrated to lower the nitrogen digestibility in broilers fed on 250g/kg (33). Recently, it has been considered a good dietary source of natural antioxidants (16). Antioxidant levels were assessed by the oxygen radical absorbance capacity, and found higher in the raw than in cooked leaf extracts. In the previous publication of Kuti and Konuru (16), flavonoid glycosides of kaempferol and quercetin, respectively, accounted for 77% and 23%, in the raw leaves of this plant.

Further investigations on the medicinal potentials of this plant are needed for a number of reasons. First, in the United States there is a rising trend in the consumption of botanical products as alternative approaches for good health maintenance in preference to the use of synthetic drugs (19). This has been due to complex changes in the health care system and the increase in self medication among patients (3, 14). Secondly, there is no magic bullet to treat diseases. It is now becoming evident that diseases are polymorphic in nature and that multiple treatment approaches may have increased effectiveness (20). Thirdly, many of the therapies considered in Western countries as alternative and/or complementary, are the mainstream of developing countries and minorities in the west, where, by contrast, modern allopathic drugs are alternative. Fourthly, diabetes is one of the diseases seriously affecting the minority groups in the United States of America. Diabetes affects about 18.2 million people in the United States (19). It is estimated that it will increase by 165% to 29 million people in the year 2050 (19). More than 2.8 million African Americans suffer from diabetes while death rates are 27% higher for blacks than for whites (19). While diabetes occurs among the people of all ages and races, some groups like the African-Americans, Hispanics, Native Americans, and Asian Americans/Pacific Islanders have a higher risk for developing Type 2 diabetes (19). This disparity affects the African-Americans 1.6 times more than the other racial groups (22, 23). Most AA adults (90-95%) have Type II diabetes; a disease caused by the body's resistance to the action of insulin and impaired insulin secretion (22). Type I diabetes is also common in a small number of African-Americans children, American-Indians and Hispanics but lower for white American children.

Diabetes is producing important health care issues. For example, increase in diabetes is also associated with a number of symptoms such as blindness and neuropathies. It is estimated that over fifty percent of those with diabetes would some form of peripheral neuropathy. With the development of such symptoms the quality of life and treatments become more difficult. This strongly supports an approach of prevention to minimize the occurrence of the disease. If the disease occurs, it is important to control the blood sugar levels by keeping it low. This approach needs alternative treatment options.

Moreover, there is a global increase in the incidence of DM and this has led to the emergence of CAM practices utilizing botanicals to treat DM and other diseases (41). Diabetes can be treated with diet, insulin and hypoglycemic agents that could be synthetic or of herbal origin. The effectiveness with these different approaches varies. There are many oral hypoglycemic agents available for example: Guanides, e.g. synthalin A/B, Sulphonylureas, e.g. chlorpropamide (Diabenese), Biguanides, e.g. metformin (Glucophage), Alpha-Glucosidase inhibitors, e.g. acarbose (Precose), Thiazolidinediones, e.g., rosiglitazone (Anandia), and Benzoic acid analogs, e.g., repaglinide (Prandin) (28). However, the resistance to insulin action with associated complications such as blindness in adults, renal failure, non-traumatic amputations, adverse reactions from other synthetic hypoglycemic agents, poor accessibility to official health system, trust on traditional healers, and high cost have necessitated the search for herbal alternatives (22).

In view of the above, the potential of herbal agents continues to be explored. Many

plant species are in use for herbal treatment of diabetes. For example, a decoction of the root of *Vernonia amygdalina* and the consumption of the leaves as vegetables has been found to exert blood glucose lowering effect in diabetic humans, (Makinde, Personal Communication, 2003). Some herbal products from different parts of the world have been suggested to have great potential for use as chemopreventive and/or chemotherapeutic agents for DM in rats (1, 4, 7, 14, 29). Quite a number of plant extracts have also been demonstrated to have a significant lowering of glucose level and insulin secretion in non-insulin dependent type 2 diabetic mice (8, 14, 25, 35). In a related work, green tea, has been suggested epidemiologically to prevent type 2 diabetes in humans and mice by improving on glucose metabolism (37). *C. aconitifolius* plant extract is another folk medicine which is considered an effective remedy for type II diabetes in southwestern Nigeria. The traditional healer's claim of the clinical efficacy of the ground, dried leaves of CA has never been subjected to scientific evaluation. It is therefore, the purpose of this study to investigate the anti-diabetic effect of *C. aconitifolius (Euphorbiaceae).* In this paper, we report the effectiveness of CA leaf extract on blood glucose and insulin levels in type 2 inbred diabetic mice as well as the histology of the pancreas, liver and kidney tissues.

MATERIALS AND METHODS

Reagents

ACCU-CHEK Comfort Curve Glucose test strips and Chlorpropamide (ICN Biochemicals Inc.) were purchased from Fischer Scientific, Inc., Hanover Park, IL 60133 while Mouse Insulin Elisa Kits were purchased from ALPCO Diagnostics, Windham, NH 03087.

Plant material

Fresh leaves of CA were collected during the rainy season from southwestern Nigeria, and identified using descriptions in literature (6). Authentication was done in the Department of Pharmacognosy, University of Ibadan, and voucher number (FHI 105343) obtained from the Nigeria Forestry Research Institute, Ibadan, Nigeria. The plant material was later carefully air-dried indoor at room temperature for three days before drying in oven between 40-50ºC; then comminuted into moderately coarse powder with an electric blender and kept in sealed polythene bags till animal studies. In addition, a nursery of the plant is being grown at MSU Greenhouse. This is part of the standardization measure that will ensure common tropical temperature, watering, fertilization, and harvest conditions.

Preparation of Plant Extracts

Standard extraction methods (14) were used. The powdered leaves (50 g) were dried for 10 min. at 50 °C and extracted with 250 mL de-ionized distilled water. The filtrate was later concentrated to dryness with a suitable freeze-dryer (Virtis-Sentry Co. Inc., N.Y 12525). The crude dried extracts were then weighed and calculated as per dried weight. They were kept in airtight plastic bottles inside desiccators until ready for use. A mass of 0.01 g crude extract per 0.05 mL water was used as stock solution for the mice studies.

Mice studies

Inbred type 2 diabetic mice (KK/HIJ, Stock Number 002106) purchased from The Jackson Laboratory in Bar Harbor, ME 04609 were used. The KK/HlJ strain animals were male mice which exhibit diabetic symptoms that include hyperglycemia, hyperinsulinemia, and insulin resistance. This strains serves as a model of non-insulin dependent diabetes mellitus, type 2. They were 4-8 weeks old and weighed on average 25.0 g. Before undergoing any experimentation the mice were quarantined and allowed four weeks to acclimatize to our animal house facility. The mice were fed on a standard pellet diet (23% protein, 4% fat) purchased from Quality Laboratory Products, Elkridge, MD 21057 and allowed access to water *ad libitum* in a humidity and temperature-controlled environment. Our experiments were conducted in full compliance with Morgan State University IRB (MSU IACUC# MO-03-B-309).

Baseline blood glucose levels and weights of the mice were determined before treatment. The mice were divided into four groups of five animals each (Groups I – IV). Group I served as control, Group II was the reference while Groups III and IV were designated as the test groups A and B. All the mice were weighed regularly, fed standard pellet diet and had access to water *ad libitum*. After an overnight fast, Group I (the control group), received no treatment. Group II (reference group) received chlorpropamide at a dose of 1.0 mg/kg body weight while Groups III and IV (test groups A and B) received 1.0 g/kg body weight of the plant extract. Blood glucose levels were measured as described earlier (13) with AccuData GTS glucometer (Boehringer Mannheim Corporation, Indianapolis, IN 46256). Blood glucose and plasma insulin levels were taken before and after two hours of treatment. Plasma insulin was assayed by using ultra-sensitive mouse insulin ELISA kit from Alpco Diagnostic, Windham, NH 03087.

Histological evaluation of plant extract on pancreas, liver and kidney

The histology of the selected organs was viewed in the non-treated and the treated (KK/HIJ) mice (N=5). The mice were put in a state of euthanasia (painless death) by CO_2 and sacrificed by cervical dislocation. Standard tissue fixation method of Reid *et al* (32) was employed. The necessary organs (pancreas, liver and kidney) were removed and fixed by submersion in formalin. After fixation, the tissue was embedded in paraffin in the standard fashion. Six micron thick sections were produced. Hematoxalin and Eosin (H&E) stain was used for morphological analysis. Photographs were taken of representative areas.

Microscopic analysis

H&E sections were observed for the pancreas, liver and kidney. Comparisons were made between the control and treatment groups. At this stage, the treated groups were compared to the untreated groups whose pathology typically demonstrates lack of Islets in the pancreas; inflammation and vaculation in the liver and alteration and loss of the glomeruli in the kidney.

Statistical analysis

Student's T-test (5) was used for statistical analysis where P values of less than 0.05 were considered significant. The blood glucose and plasma insulin levels in the various groups were expressed as mean ± s.d.

RESULTS

Table 1, Figures 1 and 2 illustrate the blood glucose and plasma insulin levels among the different groups of inbred type 2 diabetic mice initially at 0 hr and 2 hrs after treatment. Groups I – III were moderately diabetic with blood glucose levels of 100 – 300 mg/dL; while Group IV mice were severely diabetic as their blood glucose level was above 300 mg/dL (2). CA and chlorpropamide decreased by 25.6% and 16.3% respectively, the blood glucose levels for the moderately diabetic mice. The blood glucose levels of the severely diabetic mice were lowered by 43.7% using the same dose of CA as for the moderately diabetic mice. In the control group, there was no statistically significant difference between the initial blood glucose level and the level after two hours. The difference was however statistically significant for Group II ($P<0.0002$), Group III ($P<0.002$) and Group IV ($P<0.0001$). The difference in the insulin levels for Group II, III and IV, initial and after two hours, were statistically significant ($P<0.05$).

Typical microscopic changes can be found in Figure 3. The pancreas, liver and kidney tissues present with inflammatory changes in both treatment groups. The inflammation is more severe in the untreated animals (Group I). There was almost complete ablation of the islets of Langerhans cells in the pancreas of untreated animals (Group I). There was substantial preservation of the islets in the pancreas of the treated animals (Group III). There was some multi-focal fatty degeneration of the liver in both groups. In the kidney, there was destruction of the glomeruli in the untreated animals (Group I). But intact ones were identified in the treated animals (Group III).

Table 1. Blood Glucose and Plasma Insulin Levels (n = 5, mean ± s.d.) of Type 2 Inbred Diabetic Mice

Group		Blood Glucose Levels (mg/dL)		Plasma Insulin Levels (µg/mL)	
		0 hr	2 hr	0 hr	2 hr
I	Control	219.0±14.2	212.4±12.7	221.9±79.8	378.7±57.0
II	Reference	211.4±17.2	157.2±11.6*	267.2±91.3	689.8±150.7^{+}
III	Test group A	207.0±8.9	173.2±17.3**	201.7±88.4	596.1±174.3^{+}
IV	Test group B	384.4±55.6	216.4±56.0***	151.5±63.3	634.5±142.9^{+}

NB: I. Moderately diabetic mice un-treated with 1.0 g/kg b.w. of plant extract

II. Moderately diabetic mice treated with 1.0 mg/kg b.w. of chlorpropamide

III. Moderately diabetic mice treated with 1.0 g/kg b.w. of plant extract

IV. Severely diabetic mice treated with 1.0 g/kg b.w. of plant extract

*$P<0.0002$ compared with the initial blood glucose level (0 hr) for the Reference group

**$P<0.002$ compared with the initial blood glucose level (0 hr) for test group A

***$P<0.0001$ compared with the initial blood glucose level (0 hr) for test group B

$^{+}P<0.05$ compared with the initial plasma insulin level (0 hr) in the respective group.

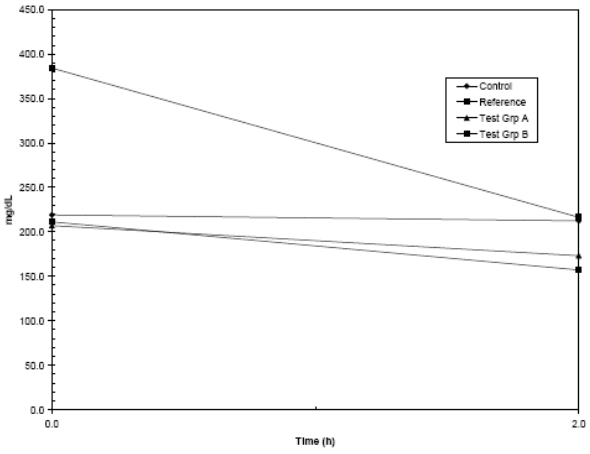

Figure 1. Blood Glucose Levels
Control, moderately diabetic mice un-treated with 1.0 g/kg b.w. of plant extract
Reference, moderately diabetic mice treated with 1.0 mg/kg b.w. of chlorpropamide
Test Group A, moderately diabetic mice treated with 1.0 g/kg b.w. of plant extract
Test Group B, severely diabetic mice treated with 1.0 g/kg b.w. of plant extract

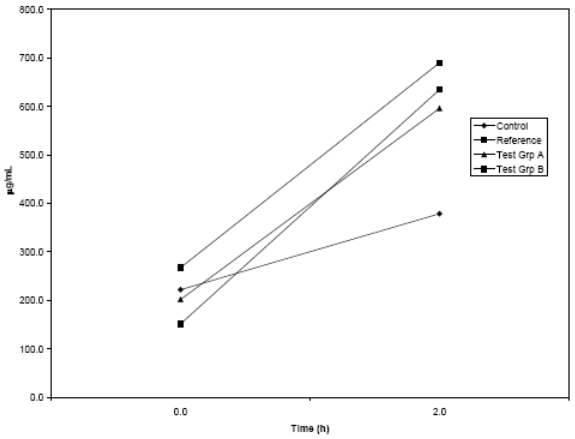

Figure 2. Plasma Insulin Levels
Control, moderately diabetic mice un-treated with 1.0 g/kg b.w. of plant extract
Reference, moderately diabetic mice treated with 1.0 mg/kg b.w. of chlorpropamide
Test Group A, moderately diabetic mice treated with 1.0 g/kg b.w. of plant extract
Test Group B, severely diabetic mice treated with 1.0 g/kg b.w. of plant extract

DISCUSSION

Traditional healers in various countries have used CA for the suppression of glucose levels in diabetic individuals. In southwestern Nigeria traditional healers use CA as a therapeutic agent for treating DM. It is also being used in Mexico and Florida, USA. Its use in these areas is apparently increasing. One contributing factor for this effect may be lack of access to medical care (4, 9). The increased use of this herb requires that it be investigated for its effectiveness, side effects and toxicity. Before such comprehensive studies can be justified, it is necessary to establish if it does have an effect on insulin levels. This present study was therefore carried out to evaluate the effect of CA leaf extracts on blood glucose levels and plasma insulin in inbred diabetic mice (KK/HIJ).

Consistent with our previous studies, using alloxan-induced diabetic mice (14) the blood glucose levels decreased significantly, in both the moderately and the severely inbred diabetic mice that were treated with the CA. The result was also consistent with the earlier works on the suppression of glucose uptake and elevation of plasma insulin with *C. cassiae* and *C. indica* respectively, using non-insulin dependent type II diabetic mice (15) and rat (38). Present studies showed that 1.0g/kg of CA lowered the blood glucose levels and elevated those of plasma insulin at a comparative rate to chlorpropamide, a reference drug. This hypoglycemic behavior of CA could be due to the insulinogenic activity that possibly stimulates insulin secretion from the dormant β-cells and/or from regenerated β-cells. Other studies on *M. charantia (*7) and *M. cymbalaria* botanicals have observed a similar insulinogenic activity in alloxan-diabetic rats (16). These results suggest that CA has a regulatory role in blood glucose level and it may also exert a blood glucose-suppressing effect by improving insulin sensitivity or slowing absorption of carbohydrates in the small intestine.

The histological effect of CA on pancreas, liver and kidney tissues was also investigated. While the changes were not quantified they are consistent with the above observations of insulin and glucose levels. Future studies will determine if islet preservation is in fact occurring and the result of treatment. The pathology in the liver and kidney is a type found in this experimental animal model. The mechanism for the apparent decrease in pathology in the treated animals (Group III) is not known. We believe that the plant extract did not produce any identifiable pathology with its present dose. But, in view of the interesting results, a more extensive analysis of organs in normal controls (non diabetic animals) is needed to determine if there are any pathological changes with higher doses and with different time frames, animal age, etc. Gene chip analysis may be informative in identifying what genes may be altered by the treatment.

These results are consistent with the view that CA has a hypoglycemic effect like chlorpropamide. This is a promising observation for CA as it may offer potential as a new treatment for diabetes management/treatment. A possible drawback is that the CA extract may exert broad effects that are not specific (39). However, the etiology of type II diabetes is so complex that, even if the CA has no effect on a specific target/receptor, it may have indirect effects on anti-diabetic mechanisms.

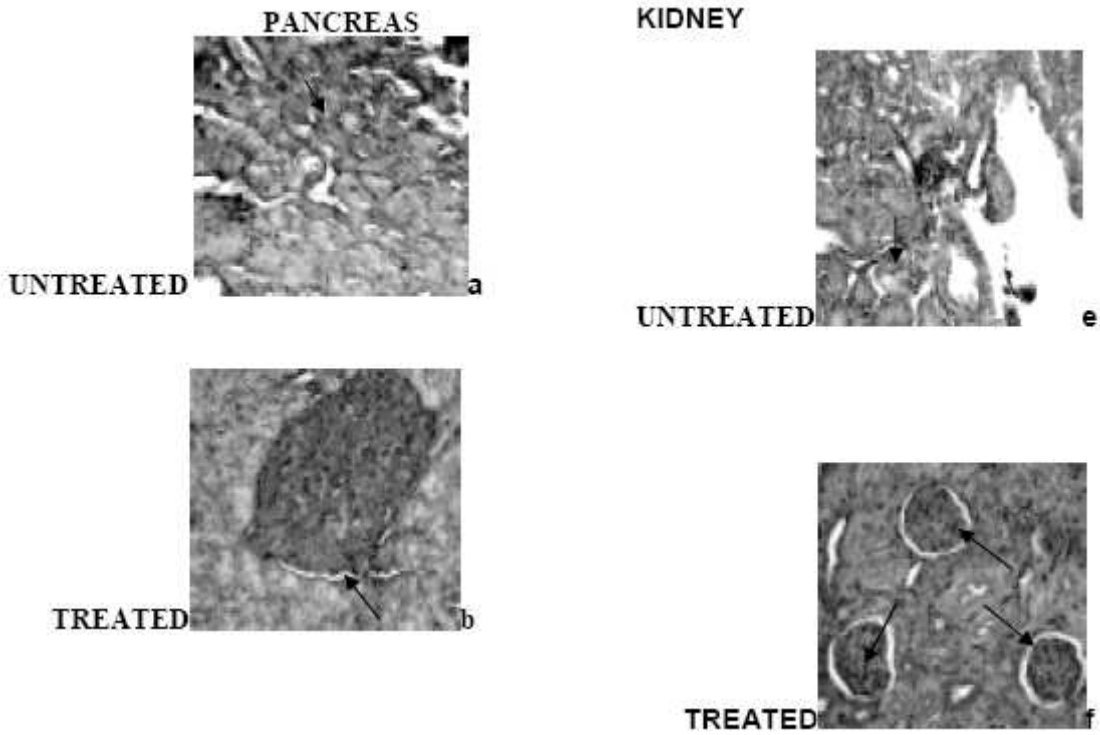

Figure 3. Histological pictures of pancreas, Liver and Kidney at original magnification 20X.
The micrographs are representative H&E preparations of untreated (a, c, e) and treated (b, d, f) groups.
The pancreas in the untreated group (a) typically displays lack of Islets of Langerhans while the treated (b) show preservation of these. Arrow demonstrates normal Islet of Langerhans.
Both untreated (c) and treated groups (d) display some inflammatory changes and fatty degeneration. But the untreated group shows much more of these changes.
Both groups (e & f) display inflammatory change and destruction of glomeruli but the treated (f) group shows marked preservation for these structures. See arrows for examples of preserved glomeruli.

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