Phytochemical and Micronutrient Composition of Anacardium Occidentale Linn (cashew) stem-bark hydroethanolic extract and its effect on the fasting blood glucose levels and body weight of diabetic wistar rats

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Citation

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Abstract

Anacardium occidentale L. stem-bark extract is used in some parts of Nigeria to treat diabetes. This study is aimed at validating the anti-diabetic property of these plants, its effect on body weight and also to screen for the presence of any bioactive component of the plant that may be responsible for any anti-diabetic effect. Twenty-four presumably healthy wistar rats of average weight 150g were randomly distributed into four groups (A, B, C and D) of 6 rats each. A single intraperitoneal dose of 65mg/kgbwt of streptozotocin was used to induce experimental diabetes in rats in groups B, C and D while group A was left non-diabetic. Groups A and B served as the negative and positive control groups respectively and received 500mg/kgbwt of Anacardium occidentale stem-bark extract and group D received 5IU of insulin. Groups A, C and D showed significant decrease in fasting blood sugar ($p<.05$) while in group B (positive control) the drop in fasting blood sugar for statistically insignificant. Groups A, C and D showed significant weight gain ($p<.05$) while Group B showed a significant weight loss ($p<.05$). Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, saponins, phenols, oxalate and phytate while the micronutrient composition included some vitamins (A, B, B2, B3 and C) and some minerals (Na, K, Ca, Mg, P, Fe, Cu and Se). Proximate composition revealed the presence of protein, carbohydrate, fat and fibre.

INTRODUCTION

There are over 150 million people with diabetes mellitus worldwide (Moller and Filler, 1991). The frequency may escalate, with a major impact on the population of developing countries due to absence of effective and affordable interventions of diabetes mellitus (Marx, 2002). The search for anti-diabetic agents has been focused on plants because of their availability, effectiveness, affordability, and probable low side effects (Marles and Farnsworth, 2005). Traditional medicinal plants with various active principles and properties have been used since ancient times to treat a great variety of human diseases such as diabetes mellitus. The beneficial multiple activities like altering carbohydrate digestion and absorption (Tiwari and Rao, 2002; Nelson et al., 1991), stimulating beta cells (Shanmugasundaram et al., 1990; Abdel et al., 1997 and Chakravathy et al., 1980) mimicking the actions of the insulin (Collier et al., 1987), inhibiting mopping up reactive oxygen species (Tiwari and Rao, 2002) present in medicinal plants account for their anti-diabetic effects. Some herbal preparations contain important micronutrients that may have favourable effects on glycaemic control and body weight (Yeh et al., 2003)

The pathogenesis of diabetes mellitus is multifactorial and demands multi-modal therapeutic approach. Medical nutrition therapy is a cornerstone in the management of diabetes though several areas of uncertainty in the dietary guidelines still exist (Franz et al., 2002)

The common denominator in diabetes mellitus is elevated fasting and postprandial blood glucose levels. Elevated blood glucose (hyperglycaemia) per se does not cause diabetic complications. It is rather the detrimental effect of glucose toxicity due to chronic hyperglycaemia, which is mediated and complicated through oxidative stress (Tiwari and Rao, 2002). The pancreas has a relatively weak intrinsic defense system against oxidative stress (Tiedge et al., 1997) and therefore the defense needs to be externally strengthened

to be able to combat the chronic hyperglycaemia so the need for adjuvant nutritional therapy.

MATERIALS AND METHODS

Experimental design: Twenty-four presumably healthy wistar rats of both sexes weighing between 150g to 155g were used in this study. The rats were randomly grouped into four groups of six rats each (A, B, C, and D). The male and female rats were put in separate cages.

Experimental diabetes was induced using a single intraperitoneal injection of 65mg/kg body weight of streptozotocin in rats in groups B, C and D after an overnight fast. All the rats were fed with normal rat chow and given water freely.

760g of Anacardium Occidentale L. powder was soaked in 1.5litres of 80% ethanol and homogenized using an electric blender. The homogenate was allowed in the refrigerator at 4ºC for 48hours. The mixture was then filtered with a chess cloth, and then with Whatman No.1 filter paper. The homogenous filtrate got was concentrated using a rotary evaporator to about 10% of its original volume. The concentrate was allowed open in a water bath at 40ºC for complete dryness. The yield was 47.4g (6.24%) of an oily brown substance which was kept refrigerated until use. The extract was reconstituted with normal saline before administration.

Non-diabetic group A rats and diabetic group B rats received 0.4ml of normal saline. Herbal extract, insulin and normal saline administration were done. Diabetic group C rats received 500mg/kgbwt of Anacardium Occidentale stem-bark extract. Diabetic group D rats received subcutaneous injection of 5IU of insulin as used by Sonia and Srinivasan (1999). The experiment, which lasted for 28 days, was carried out in the Department of Anatomy, University of Calabar, Nigeria with the approval of the Ethics Committee of the university.

Fasting blood glucose was monitored twice weekly using one-touch ultra mini glucometer (Lifescan Inc.) Blood was collected by venepuncture of the tail vein. Body weight was measured every week. 72hours post-induction fasting blood sugar was measured and only rats with fasting blood glucose greater than 13.3mmol/l were adjudged to be diabetic (Cetto et al., 2000) and were used for this study. Quantitative proximate composition was done using methods described by Chang (2003) for percentage protein content, Kirk and Sawyer (1998) for percentage fat content, James (1995) for percentage fibre and carbohydrate contents.

Determination of quantitative micronutrient composition was also done using methods described by Kirk and Sawyer (1998) and James (1995) for Vitamins and minerals. Quantitative phytochemical analysis was done using methods described by Trease and Evans (1996), for flavonoids, saponins and alkaloids Kirk and Sawyer (1998) for tannins and AOAC (1990) for phenols

Statistical analyses: Data are represented as means ± SEM and evaluated using student's t-test. Groups were considered to be significantly different if $p<0.05$

RESULTS

Effect of Anacardium Occidentale L. stem-bark extract on fasting blood glucose levels and body weight changes.

Figure 1

Table 1 Mean values of body weight

	Non-diabetic Group A (negative control)	Diabetic Group B (positive control)	Diabetic Group C (500mg/kgbwt of AO)	Diabetic Group D (5IU of NPH)
Weight at the beginning of experiment (g)	150.00± 0.00	150.00± 0.00	150.00± 0.00	150.00± 0.00
Weight at the end of experiment (g)	170.33± 1.05	*117.5± 1.88	*163.67± 1.02	160.67± 1.02

Data represent mean ± SEM n=6 *$p<0.05$

There was a significant decrease ($p<0.05$) in body weight in Group B rats. There was a significant increase in body weight in Group C ($p<0.05$) comparing the weight at the beginning of the experiment and at the end but significant difference does not exist between this group and the negative control.

Figure 2

Table 2 Mean values of fasting blood glucose

	Non-diabetic Group A (negative control)	Diabetic Group B (positive control)	Diabetic Group C (500mg/kgbwt of AO)	Diabetic Group D (5IU of NPH)
FBG at the beginning of the experiment (mmol/l)	4.40± 0.59	24.25± 0.73	33.67± 0.62	25.67± 1.30
FBS at the end of the experiment (mmol/l)	4.20± 0.32	*21.20± 1.03	*3.97± 0.45	*4.53± 0.50

Data represent mean ± SEM n=6 *$p<0.05$

There was significant ($p<0.05$) decrease in fasting blood

sugar in diabetic groups C and D. Blood glucose returned to normal. In the diabetic group B, fasting blood sugar remained high and significantly ($p<0.05$) higher than the normal control group.

Quantitative Proximate and Phytochemical composition of Anacardium Occidentale L. stem-bark extract

Figure 3

Table 3

Percentage Proximate Composition				Percentage Phytochemical Composition				
Carbo-Hydrate	Protein	Fat	Fibre	Alkaloids	Flavo-noids	Saponin	Tannins	Phenols
15.72± 0.42	6.77± 0.14	4.13± 0.01	22.65± 0.07	1.57± 0.03	0.39± 0.02	0.43± 0.01	0.67± 0.01	0.19± 0.01

Data represents mean ± SEM n=3

Proximate composition analysis revealed the presence of carbohydrates, proteins, fat and fibre.

Phytochemical analypsis revealed the presence of alkaloids, flavonoids, saponins, tannins and phenols.

Figure 4

Table 4

Vitamins (mg/100g)					Minerals (mg/100g)							
A	B1	B2	B3	C	Na^+	K^+	Ca^{2+}	Mg^{2+}	P	Fe	Cu	Se
11.05 ±0.12	0.03± 0.01	0.05± 0.01	0.15± 0.01	166.60 ±1.47	33.05 ±0.11	179.30 ±0.58	232.46 ±2.83	37.20 ±1.10	111.87 ±0.38	0.39 ±0.02	0.01± 0.004	0.01± 0.004

Micronutrient Composition of Anacardium occidentale Linn stem-bark extract

Data represent mean ± SEM n=3

Vitamins A, B and C were found to be present in the extract

Minerals found plant extract include Na^+, K^+, Ca^{2+}, Mg^{2+}, P, Fe, Cu and Se

DISCUSSION

The hydroethanolic extract of Anacardium occidentale Linn stem-bark was evaluated for possible presence of anti-diabetic components.

The effect of this plant extract on fasting blood glucose was evaluated using a glucometer. The extract was found to restore normal glycaemia. Hyperglycaemia per se does not cause diabetic complications. It is rather the detrimental effect of glucose toxicity due to chronic hyperglycaemia, which is mediated and complicated through oxidative stress (Tiwari and Rao, 2002)

Oxidative stress is responsible for molecular and cellular tissue damage in a wide spectrum of human diseases (Halliwell, 1994). Oxidative stress is present in type 1 diabetes (Ceriello et al., 1991) due to several mechanisms, including glucose auto-oxidation and non-enzymatic protein glycation (Sakurai and Tsuchiya, 1988; Wolf, 1993). Supportive therapy aimed at oxidative stress may help to prevent clinical complications in diabetic patients. Induction of diabetes using streptozotocin results in the generation of reactive oxygen species (Mazunder et al., 2005). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Hills, 1952)

Anti-diabetic properties and the body weight changes observed in the animals with administration of Anacardium occidentale L. stem-bark extract may be due to the presence of some micronutrients, some secondary metabolites and some food substances in it.

Flavonoids, alkaloids and saponins which are present in Anacardium occidentale L. stem-bark have been documented to have anti-oxidant effects (Olaleye et al., 2007) blood glucose reduction effect (Bolkent et al., 2000; Diatewa et al., 2004) and enhance natural resistance and recuperative powers of the body (Singh et al., 1991). People with uncontrolled diabetes are prone to develop deficiencies in some minerals, notably potassium, magnesium and zinc (Mooradian et al., 1994; Mooradian 1999) and this may predispose to carbohydrate intolerance (Chehade et al., 2009). The presence of some minerals in the plant extract is a good micronutrient supplement because they will help in modulating the immune system and pancreatic insulin secretion and action (Holick, 2007 and Rosen, 2005)

Several micronutrients present in Anacardium occidentale Linn stem-bark have potent antioxidant properties. These include Vitamin C, selenium, Vitamin A and B_3 which has also been documented to preserve beta cells mass (Visalli et al., 1999)

Studies are replete supporting significant weight reductions in untreated diabetic rat models (Nwanjo, 2005; Atangwo et al., 2007; Ahmed et al., 2005 and Kechrid and Bouzena, 2004). This was also the case in this study. In the treated diabetic group however, weight gain was similar to the

negative control group which suggest a relationship between glycaemic control and weight gain. The herb has an antihyperglycaemic effect and the groups that achieved euglycaemia also had an improvement in their weight gain.

In conclusion, Anacardium occidentale L. stem-bark extract has antihyperglycaemic property and positive effect on weight gain and these actions may be attributed to the multiple physiological effects of the micronutrient and phytochemical composition of the herb.

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