# Biochemical Changes in Non-Diabetic and Diabetic Wistar Rats with the Administration of Stem-Bark Extract of *Anacardium occidentale* Linn (Cashew).

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## ABSTRACT

In this study, forty-eight presumably healthy Wistar rats of both sexes weighing between 150-160g were used. The animals were divided into two groups (N and D). Diabetes was induced with 65mg/kg body weight of streptozotocin intraperitoneally in group D animals. Diabetes was confirmed after 72 hours of induction using a glucometer. *Anacardium occidentale* Linn stem-bark was extracted using the method described by Ugochukwu and Babady (2003).The diabetic and non-diabetic rats were randomly divided into four parallel groups of six rats each. The control groups received normal saline orally daily while the other groups received varied oral doses of the stem-bark extract daily. After 28 days of treatment, the animals were anaesthetized. Blood samples for analysis was collected via cardiac puncture. Results revealed that the alcoholic stem-bark extract of *Anacardium occidentale* Linn was able to increase insulin levels significantly ($p < 0.05$) in diabetic rats in a dose dependent manner but had no effect on the normal rats. The extract did not seem to have any deleterious effect on the serum activities of liver enzymes and urea, electrolyte and creatinine concentrations of non-diabetic rats and so may be a good alternative in diabetic management if adequately explored.

(Keywords: *Anacardium occidentale* Linn, cashew extract, diabetes, streptozotocin, biochemical parameters, Wistar rats)

## INTRODUCTION

Diabetes has become a serious health challenge the world over, with a major impact on the population of developing countries due to absence of curative and affordable interventions. People in all continents have used hundreds to thousands of indigenous plants for the treatment of ailments since prehistoric times. The use of herbs to treat disease is almost universal among non-industrialized society (DaSilva *et. al.,* 2002). In developing countries, poverty and dwindling economic resources make people resort to these cheap resources for their immediate needs.

Diabetes mellitus is a chronic disorder and for now there is no substantive cure. Its management is cumbersome and expensive. Pharmaceuticals are prohibitively expensive for most of the world's population, as a result many patients leave the hospitals against medical advice to resort to the use of plant materials.

A wide variety of plant materials have been used by traditional medicine practitioners to manage diabetes mellitus. Among the plant materials used in treating diabetes mellitus in some parts of Nigeria is *Anacardium Occidentale* Linn (cashew). *Anacardium occidentale* L. belongs to the family of Anacardiacae, genus Anacardium and species occidentale the commonly used parts are the leaves, bark, fruit and the nut. The leaves and the stem-bark extracts have been experimentally tested (Tedong *et. al.,* 2005, Eliakim-Ikechukwu and Obri, 2010) and found to have hypoglycaemic effect. The stem-bark extract has also been found to have regenerative effect on the pancreatic islet beta cells (Bassey *et. al.,* 2012). Though this herb has been used widely in folk medicine for the management of diabetes mellitus and experimentally proven to have beneficial effects in the management of diabetes mellitus, its effect on the biochemical processes in the body has not been documented. This study seeks to address this.

## MATERIALS AND METHODS

Fresh stem-bark of *Anacardium occidentale* L. was collected and dried and then blended into fine powder. It was soaked in 80% ethanol and homogenized using an electric blender. The homogenate was kept in the refrigerator at $4^0$C for 48 hours. The mixture was filtered. The homogenous filtrate obtained was concentrated using a rotary evaporator. The concentrate was allowed open in a water bath at $40^0$C for complete dryness yielding an oily brown substance. The extract was reconstituted with normal saline before administration.

Forty-eight presumably healthy Wistar rats of both sexes were used for this study. The Wistar rats weighing between 150g-160g were housed in well ventilated wooden cages wit netted covers and saw dust beddings. The beddings were changed every other day to maintain a suitable hygienic environment. The rats were fed with pellets and given water freely.

The Wistar rats were broadly divided into two groups, 'N' for non-diabetic and 'D' for diabetic sub groups. Groups N and D were divided randomly into four parallel subgroups of non-diabetic ($A_N$, $B_N$, $C_N$, $D_N$) and diabetic ($A_D$, $B_D$, $C_D$, and $D_D$) pairs of six animals each.

Diabetes mellitus was induced using a single intraperitoneal injection of streptozotocin reconstituted in normal saline after an overnight fast. Fasting blood sugar (FBS) was measured 72 hours after the induction and rats with FBS greater than 240mg/dl (>13.3mmol/l) were judged to be diabetic and used for the study.

Animals in groups $A_N$ and $A_D$ served as non-diabetic and diabetic groups control respectively and each received oral doses of 0.4 ml of normal saline daily. Animals in groups $B_N$ and $B_D$ received oral doses of 300mg/kg body weight daily of *Anacardium occidentale* L. stem-bark extract Animals in groups $C_N$ and $C_D$ received oral doses of 500mg/kg body weight daily of *Anacardium occidentale* L. stem-bark extract while animals in groups $D_N$ and $D_D$ received subcutaneous injections of 5iu/kg body weight daily of soluble insulin to simulate the human regimen. The experiment lasted for twenty-eight days.

At the end of the experiment, the animals were put on an overnight fast and thereafter they were anaesthetized using chloroform inhalation. The thoracic cage was opened up to expose the heart, cardiac puncture was done and blood collected into plain bottles for biochemical analysis.

Urea estimation was done using Urease-Berthelot method (Weatherburn, 1967), serum sodium concentration was done using colorimetric method (Maruna, 1958), serum potassium concentration was done using photometric turbidimetric test, serum bicarbonate concentration was measured using carbondioxide reagent set (Kaplan and Pesece, 1984), serum chloride concentration was done using mercuric thiocyanate single reagent kit (Tietz, 1976), serum creatinine was determined using direct endpoint method (Hienegard and Tiderstrom, 1973; Buffer, 1975), serum alanine and aspartate aminotransferases determination was done using the principle described by Reitman and Frankel, 1957) and quantitative determination of anti-insulin in serum was done using Microwell method of Dialab.

## RESULTS

The results are presented in the tales below.

### Effect of *Anacardium occidentale* Linn Stem-Bark Alcoholic Extract on Serum Urea, Electrolyte and Creatinine

Serum urea concentration showed some variations that did not follow any particular pattern and the differences were not statistically significant ($p<0.05$)

The serum creatinine values did not show any significant variation ($P<0.05$) in both the diabetic and non-diabetic groups. The serum electrolyte profile also did not show any significant difference ($P<0.05$) between the rats that received varied doses of *Anacardium occidentale* Linn stem-bark extract and those that did not.

Results from this study suggests that *Anacardium occidentale* Linn stem-bark extract may not have any adverse effect on the serum urea, electrolyte and creatinine levels in non-diabetic and diabetic Wistar rats.

**Table 1:** Showing the Serum Levels of Urea, Electrolyte and Creatinine of Non-Diabetic and Diabetic Wistar Rats.

| Serum concentrations | NON-DIABETIC GROUPS | | | | DIABETIC GROUPS | | | |
|---|---|---|---|---|---|---|---|---|
| | $A_N$ 0.4ml NS/d | $B_N$ 300mg/kg.b wt. *AO*/d | $C_N$ 500mg/kg. bwt. *AO*/d | $D_N$ 5iu/kg. bwt. Insulin/d | $A_D$ 0.4ml NS/d. | $B_D$ 300mg/kg. bwt.*AO*/d | $C_D$ 500mg/kg.b wt. *AO*/d | $D_D$ 5iu/kg. bwt. insulin/d |
| urea (mmol/l) | 10.45± 1.87 | 9.45± 0.64 | 5.77± 0.45 | 11.53± 0.62 | 10.64± 1.26 | 9.31± 0.37 | 9.16± 0.38 | 8.27± 1.31 |
| creatinine (mg/dl) | 1.79± 0.50 | 1.19± 0.29 | 1.79± 0.50 | 1.43± 0.50 | 1.67± 0.77 | 1.00± 0.20 | 1.43± 0.05 | 1.79± 0.35 |
| $Na^+$ (mmol/l) | 126.50± 0.71 | 129.33± 1.08 | 131.50± 0.71 | 129.33± 1.47 | 126.33± 0.82 | 128.25± 0.25 | 128.33± 0.82 | 128.50± 0.71 |
| $K^+$ (mmol/l) | 4.70± 0.14 | 4.83± 0.99 | 5.85± 0.92 | 4.90± 0.38 | 4.30± 0.14 | 3.45± 1.13 | 3.70± 0.26 | 3.55± 0.35 |
| $HCO_3^-$ (mmol/l) | 18.25± 0.35 | 18.17± 0.21 | 19.00± 1.41 | 18.83± 1.22 | 18.75± 0.55 | 18.50± 0.14 | 18.75± 0.35 | 18.35± 0.35 |
| $Cl^-$ (mmol/l) | 95.07± 0.12 | 94.36± 1.32 | 97.18± 0.09 | 96.24± 0.57 | 95.30± 0.28 | 94.50± 0.30 | 94.36± 0.49 | 94.72± 2.69 |

Data represent mean ± SEM. n=5; NS- normal saline; *AO- Anacardium occidentale;* bwt- body weight; d- day.

**Table 2:** Showing Mean Serum Levels of Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) in Non-Diabetic and Diabetic Wistar Rats.

| SERUM CONCENTRATION | NON-DIABETIC GROUPS | | | | DIABETIC GROUPS | | | |
|---|---|---|---|---|---|---|---|---|
| | $A_N$ | $B_N$ | $C_N$ | $D_N$ | $A_D$ | $B_D$ | $C_D$ | $D_D$ |
| ALT (U/L) | 15.00± 0.00 | 14.33± 0.82 | 16.00± 1.41 | 16.67± 1.08 | 14.33± 0.82 | 16.00± 0.71 | 13.00± 1.14 | 14.00± 1.41 |
| AST (U/L) | 31.00± 0.00 | 28.33± 1.63 | 33.50± 3.54 | 39.67± 2.77 | 25.67± 1.63 | 28.60± 1.10 | 36.00± 3.54 | 29.00± 2.83 |
| AST/ALT RATIO | 2.07± 0.00 | 2.00± 0.24 | 2.10± 0.04 | 2.41± 0.47 | 1.79± 0.01 | 1.80± 1.10 | 2.84± 0.55 | 2.08± 0.01 |

Data represent mean ± SEM. n=5

**Table 3:** Showing Mean Serum Insulin Levels in NonDiabetic and Diabetic Wistar Rats.

| | NON-DIABETIC GROUPS | | | | DIABETIC GROUPS | | | |
|---|---|---|---|---|---|---|---|---|
| | $A_N$ | $B_N$ | $C_N$ | $D_N$ | $A_D$ | $B_D$ | $C_D$ | $D_D$ |
| INSULIN LEVEL (µIU/ml) | 18.12± 2.32 | 17.87± 3.52 | 18.53± 2.67 | 19.95± 0.67 | 3.37± 17.50 | 13.50± 8.80 | 30.95± 9.14 | 10.4± 2.76 |

Date represent mean ± SEM. N=3

## Serum Liver Function Indices

Table 2 revealed significant decrease in serum AST activity and in AST/ALT ratio in the diabetic control group ($P< 0.05$) when compared with non-diabetic control group slight variations in aminotransferases serum activity were also observed but not significant statistically ($P<0.05$).

## Serum Insulin Levels

Among the non-diabetic groups there was insignificant variations in the serum insulin level ($p<0.05$). In comparison with the non-diabetic control group, the serum insulin level of the diabetic control group was significantly lower at $P<0.05$. Among the diabetic groups, insulin levels of the animals that received *Anacardium occidentale* L. stem-bark extract and insulin were significantly higher ($P<0.05$).

The two diabetic groups of rats that received *Anacardium occidentale* L. stem-bark extract showed significant ($p<0.05$) dose-dependent increase in the serum insulin levels as compared with the diabetic control. There was no such increase in the non-diabetic animals.

## DISCUSSION AND CONCLUSION

The desire to achieve cure in diabetes mellitus with minimal cost and side effect has prompted several studies using locally available herbs.

In this study, some biochemical parameters were studied. Diabetes causes degenerative complications if not controlled (Conrick, 2005) thereby resulting to electrolyte derangement. However, electrolyte derangement was not seen in this study even in diabetic control group. Eliakim-Ikechukwu *et al.* (2010) reported a gradual recovery towards normoglycaemia in STZ- induced diabetic rats with time. Recent evidence from both humans (Bresson *et. al.,* 2006, Voltarelli *et. al.,* 2007) and rodents (Suri *et. al*., 2006, Melton, 2006) suggest that beta cell function can partly recover if autoimmunity is blocked.

Since STZ cannot cause complete disappearance of entire islet cells (Mahmoudzadeh-Sageb *et al.,* 2010), it is possible that the small quantity of insulin produced by the residual beta cells in the pancreatic islet is enough to protect the body from severe electrolyte imbalance. It is also possible that the duration of study may be too short as to allow for a substantive effect on the body. Normal levels of electrolyte was also seen in the non-diabetic and the diabetic groups that received the alcoholic stem-bark extract (SBE) of *Anacardium occidentale* Linn suggesting that the extract may be safe to use and may protect the body from electrolyte imbalance in diabetes.

Glomerular function can be assessed by measuring the serum levels of urea and creatinine. Twenty-five percent of patients develop diabetic nephropathy (Renu *et al.,* 2004). Serum urea and electrolyte levels did not show any significant change suggesting that alcoholic SBE of *Anacardium occidentale* Linn may be protective and safe to the kidneys. The diabetic control group also did not show any significant difference probably because diabetic nephropathy is progressive and takes time to develop (LeRoith and Rayfield, 2007) so the extent of kidney damage may not have been severe enough to cause glomerular dysfunction. However, Eidi *et al.,* (2006) has reported elevated serum urea and creatinine in a fourteen day study.

Streptozotocin has been noted to have a significant role in the alteration of liver function (Ohkuwa *et. al.,* 1995; Eidi *et. al.,* 2006). Eidi *et al.* (2006) reported elevated serum activities of ALT and AST. Elevated serum aminotransaminases have been used for markers to detect liver disease especially ALT. In this study, serum aminotransaminases did not show increased activity in all groups. This suggests that alcoholic SBE of *Anacardium occidentale* Linn did not have toxic effect on the liver of non-diabetic rats. It also suggest that the SBE may be hepatoprotective though normal levels do not rule out significant liver disease (Giboney, 2005).

Serum AST/ALT ratio is often used to trace the cause of liver disease in evaluating hepatic disorders. An increased ratio suggests active liver damage. It is a reliable marker of insulin resistance in the obese (Kawamoto *et. al.,* 2012). The low AST/ALT ratio in the diabetic control suggests the absence of insulin resistance and absence of liver damage.

Hypoglycaemic effect of alcoholic SBE of *Anacardium occidentale* Linn has been documented (Bassey *et al.,* 2012) and this was attributed to the presence of saponins and flavonoids in the plant material (Eliakim-Ikechukwu *et al.,* 2010). These bioactive substances have been reported to exhibit hypoglycaemic effect by increasing insulin release from pancreatic beta cells (Saravana and Pari, 2008; Zambare *et al.,* 2011).

In this study, a dose dependent significant increase in insulin level was recorded in the diabetic groups that received the alcoholic SBE of *Anacardium occidentale* Linn but no increase in non-diabetic groups that received the extract. This is in agreement with Eidi *et al.,* (2006) and Eidi *et al.,* (2007). Bassey *et al.,* (2012) reported regeneration of pancreatic beta cell in STZ-induced diabetes with administration of alcoholic SBE of *Anacardium occidentale* Linn. This further suggests that the regenerated beta cells are functional and may be responsible for this increase in insulin levels.

In conclusion, alcoholic SBE of *Anacardium occidentale* Linn has no adverse effect on non-

diabetic Wistar rats. It is able to induce insulin secretion in diabetic Wistar rats in a dose dependent manner but has no such effect on the non-diabetic Wistar rats. However, a more elaborate work has to be done to fully appreciate its mechanisms of action with the hope of purification for use as a cheap alternative in the treatment of diabetes.

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