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Protective and Curative Effects of *Afzelia africana* Based diet on Alloxan induced Diabetic Rats.

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ABSTRACT
The present study examines the Protective and Curative effects of Afzelia africana based diet on alloxan induced diabetic rats. Thirty two male albino rats(Rattus novergicus) were divided into two experimental groups (A, B). GroupA were fed with standard formulated diet, GroupB were fed Afzelia africana based diet for four weeks, after which the two groups were sub-divided into subgroups A_1, A_2, and B_1, B_2. Subgroups A_2, B_2 were induced with 150mg/kg body weight of alloxan by a single intraperitonial injection. Subgroups A_1, B_1 and B_2 were maintained on the same diet. Subgroup A_2 was fed Afzelia africana based diet while the experimental period lasted for two weeks. Blood was collected from the rats through tail puncture for Glucose concentration determination. Blood sample was collected via cardiac puncture to assay for Urea, Creatinine, Sodium ion(Na^+), Potassium ion(K^+) concentrations. Significant decrease ($P \leq 0.05$) were observed in blood glucose level in GroupsB compared with A at week4 before induction of diabetes, Creatinine level in GroupsA_2 compared with A_1 and GroupsB_2 compared with B_1. In conclusion, Afzelia africana based diet in addition to possessing anti-diabetic potential could be used to ameliorate kidney disorders.
Keywords: *Afzelia africana*, *Rattus novergicus*, anti-diabetic, alloxan and blood glucose

INTRODUCTION

Diabetes mellitus remains one of the major chronic disorders of carbohydrate, lipid and protein metabolism characterized by persistent elevations of fasting blood glucose equal or greater than 140mg/dl taken on at least two separate occasions, resulting from a partial or complete cessation of insulin or secretion and/or peripheral resistance to insulin action(Murray and Pizzorno, 1997). Diabetes mellitus is often associated with increased risk of pansystemic complications which include ischaemic heart disease, nephropathy, retinopathy, neuropathy, and ulceration. Diabetes mellitus could occur alone but more often co-exists with other systemic diseases such as hypertension, dyslipidaemia, ischaemic heart disease, renal diseases etc. Products of plant origin have been known to be effective sources of chemotherapeutic agents without any underlying effects. Alloxan, a cyclic urea derivative, acts as a diabetogenic agent by its ability to cause selective cytotoxicity and necrosis of the beta cells of endocrine pancreas(Rerup, 1970).

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Plants continue to be a major source of medicines, as they have been throughout human history. The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs(Dick, 2003). A medicinal plant is any plant with one or more of its organs containing substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs(Kokowaro, 1976). They are of great importance to the health of individuals and communities; the medicinal values of certain plants like *Afzelia africana* lie in some chemical substances that produce definite physiological action on the body. The most important of this bioactive constituents of plants are flavonoids, tannins, alkaloids and food plants sometimes added to foods(Kokowaro,1976).

Afzelia africana is a tree species commonly found in savanna fringing forest and drier parts of forest regions. It is commonly referred to as mahogany, kawo. apa, and akpalata in English, Hausa, Yoruba and Igbo respectively(Keay, 1989). The seed have waxy orange cup – like structure at their base and are widely used for medicinal purposes, for industrial production of soup as soup thickening ingredients(Ajah and Madubuike,1997).

The aim of this study therefore is to examine the protective and curative effects of *Afzelia africana* based diet on alloxan induced diabetic rats.

MATERIALS AND METHODS

Afzelia africana was bought from the Eastern part of Nigeria and identified in the Herbarium of Plant Science Department Ekiti State University. The seeds were roasted to aid dehulling. The roasted seeds were ground into powdered form with a blender and sundried. The flour was then kept in air tight container until required for diet composition. All chemicals used for the study were of analytical grade (ANALAR).

Animal Groupings

Thirty two (32) male albino rats (*Rattus novergicus*) with average weight of about 90-100g were used for the study. They were obtained from College of Medicine, Ekiti State University, Ado-Ekiti, Nigeria. The rats were kept in good conditions and were given normal rat feed and water *ad libitum*. They were ramdomly divided into two experimental groups (A, B) i.e 16 rats in each group. GroupA were fed with standard formulated diet, GroupB were fed *Afzelia africana* based diet (Table 1) for four weeks. After four weeks of feeding, the two groups were sub-divided into subgroups A_1, A_2, and B_1, B_2. Subgroups A_2, B_2 were induced with 150mg/kg body weight of alloxan by a single intraperitonial injection, while subgroups A_1, B_1 were not induced with alloxan. Subgroups A_1, B_1 and B_2 were maintained on the same diet. Subgroup A_2 was fed *Afzelia africana* based diet while the experimental period lasted for two weeks.

Preparation of plasma

Blood sample collected into Lithium heparin bottle was centrifuged at 3000rpm(revolution per minutes) for 10 minutes. After centrifugation, the supernatant which was the plasma was collected using a Pasteur's pipette. The plasma, thus obtained were appropriately labeled and stored in a freezer at -10ºC

until required for further analysis such as kidney function parameters(Urea , Creatinine, Sodium ion(Na^+), Potassium ion(K^+))

Table 1: Diet Composition (g/kg)

Ingredients	A	B
Corn Starch	570	570
Casein (Protein source)	200	-
Sucrose	100	100
Vitamin-Mineral mix	50	50
Cellulose	30	30
Vegetable oil	50	50
Afzelia africana	-	200

Group A- Diet composition for Control group, Group B- *Afzelia africana* based diet

Biochemical Analysis

Creatinine was determined according to the method of Jaffe, 1941. Urea concentration was determined according to the method of Talke and Schubert, 1965. Potassium and Sodium concentrations were determined using Electrolyte analyzer. Blood glucose concentration was determined by glucose oxidase method of Barham and Trinder, 1972 using a glucometer.

Statistical analysis: The results are expressed as Mean ± standard deviation. Analysis of variance was used to test for differences in the groups. Differences were considered to be statistically significant at $P<0.05$.

RESULTS AND DISCUSSION

Table 2: Blood Glucose (mmol/l) Concentration of Experimental Rats before inducton of Diabetes

	A	B
Week1	6.00±0.48[a]	5.91±0.71[a]
Week2	5.40±0.74[a]	5.39±0.88[a]
Week3	5.56±0.91[a]	5.08±1.40[a]
Week4	4.07±0.60[b]	3.21±1.87[a]

Results are expressed as mean±SD; values in the same row with different superscript are significantly different at $P \leq 0.05$.

A – Normal Control

B – Rats fed *Afzelia africana* based diet

Table 3: Blood Glucose (mmol/l) Concentration of Diabetic and Non diabetic Rats Two weeks after induction of Diabetes

	A_1	A_2	B_1	B_2
Week1	7.08±4.66[a]	4.73±0.96[a]	6.16±6.74[a]	4.93±0.54[a]
Week2	5.80±3.69[a]	4.08±0.77[a]	4.98±4.22[a]	4.07±0.91[a]

Results are expressed as mean±SD; values in the same row with different superscript are significantly different at $P \leq 0.05$.

A_1 – normal control

A_2 – diabetic rats posttreated with *Afzelia africana* based diet

B_1 – non diabetic rats fed *Afzelia africana* based diet

B_2 – diabetic rats pretreated with *Afzelia africana* based diet

Table 4: Effects of *Afzelia africana* based diet on Kidney function Parameters of Alloxan induced diabetic Rats

Parameters	A_1	A_2	B_1	B_2
Urea(mmol/l)	5.18 ± 1.36^{a}	4.32 ± 1.50^{a}	7.64 ± 1.13^{b}	6.22 ± 1.22^{ab}
Creatinine(mmol/l)	6.63 ± 0.86^{b}	4.05 ± 1.72^{a}	6.80 ± 1.72^{c}	5.90 ± 1.42^{ab}
Potassium(md/dl)	7.82 ± 1.12^{ab}	6.66 ± 1.13^{a}	8.38 ± 1.03^{b}	7.94 ± 1.52^{ab}
Sodium(mg/dl)	12.67 ± 2.43^{ab}	5.21 ± 0.73^{a}	13.11 ± 2.71^{b}	8.63 ± 3.41^{ab}

Results are expressed as mean±SD; values in the same row with different superscript are significantly different at $P \leq 0.05$.

A_1 – normal control

A_2 – diabetic rats posttreated with *Afzelia africana* based diet

B_1 – non diabetic rats fed *Afzelia africana* based diet

B_2 – diabetic rats pretreated with *Afzelia africana* based diet

Table 2 shows blood glucose concentration of experimental rats before inducton of diabetes. Non significant decrease in GroupB compared with GroupA blood glucose level was observed from first to the third week. At week4, significant decrease ($P \leq 0.05$) was observed in blood glucose level in GroupB compared with GroupA.

Blood glucose concentration of diabetic and non diabetic rats two weeks after induction of Diabetes(Table3) shows that blood glucose level non significantly decreased in GroupA_2 compared with Group A_1 from the first to the second week. Also, non significant decrease was observed in blood glucose level in GroupB_2 compared with GroupB_1 from the first to the second week.

The effects of *Afzelia africana* based diet on kidney function parameters of alloxan induced diabetic rats is as shown in Table4. Non significant decrease in urea concentration was observed in GroupA_2 compared with GroupA_1 and GroupB_2 compared with GroupB_1 in the plasma. Significant decrease ($P \leq 0.05$) was observed in Creatinine level in GroupA_2 compared with GroupA_1 and GroupB_2 compared with GroupB_1. Non significant decrease was observed in GroupA_2 compared with GroupA_1 and GroupB_2 compared with GroupB_1 in Potassium ion concentration. Non significant decrease was observed in GroupA_2 compared with GroupA_1 and GroupB_2 compared with GroupB_1 in Sodium ion concentration.

The result from Table 2 indicate that *Afzelia africana* based diet possess significant anti hyperglyceamic activity. The component(s) responsible for the observed pharmacological effects or their mechanism of action has not been determined in this study. Non significant decrease in GroupB compared with GroupA blood glucose level was observed from week1 to week3. At week4, significant decrease ($P \leq 0.05$) was observed in blood glucose level in GroupB compared with GroupA. This result is similar with findings reported by Khosla *et al*. (1995) where blood glucose level was significantly decreased after administration of Fenugreek seed powder in normal rats. The Non significant decrease and significant decrease ($P \leq 0.05$) in blood glucose as a consequence of *Afzelia africana* based diet might be due to the presence of Fibre in the seed(Onyechi *et al*., 2012). Several studies have indicated that the dietary fiber contained in seeds lowers blood glucose and improves glucose tolerance(Valette *et al*., 1984; Srivastava *et al*., 1987). The fibre of plants may interfere with Carbohydrate absorption; thus affecting blood glucose(Nelson *et al*., 1991). This also suggests that *Afzelia africana* seed may have low glycemic index and able to reduce blood glucose concentration in normal rats.

Alloxan acts as a cytotoxin for beta-cells of the islets of langerhans, causing diabetes by inducing cell necrosis(Jorns *et al*., 1997). This study as shown in Table3 indicate that blood glucose level non significantly decreased in GroupA_2 compared with Group A_1 from week1 to week2 which is in disagreement with the results of Sada *et al*.(2013); Kazunari *et al*.((2008) in which Soya bean seed supplement and administration of *Eriobotrya japonica* seed significantly decreased blood glucose level in diabetic rats. Also, non significant decrease was observed in blood glucose level in GroupB_2 compared with GroupB_1 from week1 to week2. This is in contrast with the reports of Raveendran and Thankappan, (2012) where posttreatment with *Cocos nucifera* showed a significant reduction in the blood glucose levels compared to non diabetic control while similar observation was observed with *Cocos nucifera* pretreatment. These shows that pretreatment and posttreatment with *Afzelia africana* based diet insignificantly reduced blood glucose levels in diabetic rats, indicating the antidiabetic action of *Afzelia africana* based diet. Anidiabetic activity(lowering of blood glucose levels) has been reported with methanolic root bark extract of *Afzelia africana* in alloxan diabetic mice(Odo *et al*., 2012). Studies show that treatment with phytonutrients might be an effective strategy for reducing diabetes by influencing glucose metabolism and homeostasis by mechanisms such as modulation of glucose output from liver, inhibition of carbohydrate digestion and regulating the glucose metabolizing enzymes. The possible mechanism by which *Afzelia africana* based diet reduced blood glucose concentration of the diabetic rats can be due to the ability of *Afzelia africana* seed to increase insulin secretion from pancreas, or through extra-pancreatic mechanisms like increasing peripheral utilization of glucose or decreasing glucose absorption from gut. The antioxidant and free radical scavenging of these seed as shown in previous work by Atawodi *et al*. (2014), might increase the resistant of β cells to the toxic effects of alloxan by activating antioxidant enzymes. Igwenyi *et al*. (2013) previously

reported the presence of Flavanol(tannin) in *Afzelia africana* seed which may induce insulin secretion in diabetic rats.

Kidney maintains optimum chemical composition of body fluid by acidification of urine and removal of metabolic wastes such as urea, creatinine and ions. The usual blood test which checks whether the kidneys are functioning properly measures the level of urea, creatinine and certain undissolved salts. Urea is a byproduct from protein breakdown. About 90% of urea produced is excreted via the kidney (Walmsley *et al*., 2010). In kidneys, urea is filtered out of blood by glomeruli and is partially reabsorbed with water. It was reported that diabetic individuals had higher urea levels than nondiabetic individuals(Lal *et al*., 2009). As shown in Table4, non significant decrease in urea concentration was observed in $GroupA_2$ compared with $GroupA_1$ and $GroupB_2$ compared with $GroupB_1$ in the plasma. Thus, the non significant reduction in urea level in diabetic control group treated with *Afzelia africana* based diet compared with normal control can probably be explained through a reduction in blood glucose level. It is possible to suggest that *Afzelia africana* based diet might play an important role in reducing the risk of kidney problems. This is in line with the findings of Shukla and Papiya, (2014) in which nonsignificant decrease was observed in diabetic rats treated with *Wrightia tinctoria*. The nonsignificant decrease observed in diabetic rats pretreated with *Afzelia africana* based diet compared with nondiabetic rats fed *Afzelia africana* may signify a protective potential of *Afzelia africana* based diet against kidney failure.

Creatinine which is commonly measured as an index of glomerular function (Treasure, 2003) is a waste product from muscle creatine that is used as energy source during muscle contraction. Like urea, it is excreted exclusively through the kidney. Damage to the kidney will thus render the kidney inefficient to excrete both urea and creatinine, therefore causes their accumulation in the blood (Stevens *et al*., 2006). Hence, a higher than normal level of blood urea and creatinine will indicate kidney damage. Creatinine passes into the blood stream and is usually passed out in urine. A high blood level of creatinine indicates that the kidneys are impaired. Creatinine is usually a more accurate marker of kidney function than urea. Significant decrease ($P \leq 0.05$) was observed in Creatinine level in $GroupA_2$ compared with $GroupA_1$ and $GroupB_2$ compared with $GroupB_1$. This is consistent with the report of Shukla and Papiya, (2014) where *Wrightia tinctoria* significantly reduced ($P \leq 0.05$) creatinine level in diabetic treated rats. This study has demonstrated that *Afzelia africana* based diet might improve renal function which in turn leads to reduction in Creatinine level in treated diabetic rats compared with normal control. *Afzelia africana* based diet could be protective against kidney disorders by decreasing creatinine level in $GroupB_2$ compared with $GroupB_1$ suggesting increase in glomerular filteration rate. The non significant decrease in Urea level and significant decrease ($P \leq 0.05$) in Creatinine level observed across the groups may be due to it antioxidant potential(Igwenyi *et al*., 2013). Also, it was reported that, the administration of Flax and Pumpkin seeds mixture through the diet of diabetic rats improved the renal histological alterations

induced by alloxan, which could be attributed to its antiradical/antioxidant activities(Makni *et al*., 2010).

Electrolyte is the medical term for a salt or ion in the blood or other bodily fluid that carries a charge (Syzdek and Jaroslaw, 2007). In biological systems, the main electrolytes are: Sodium (Na^+), Potassium (K^+), Calcium (Ca^{2+}), Magnesium (Mg^{2+}), Chloride (Cl^-), Hydrogen phosphate (HPO_4^{2-}), Hydrogen carbonate (HCO_3^-). The major cation in the intracellular fluid is potassium. Together with sodium, potassium maintains and influences membrane potential, except it acts from within cell walls rather than from extracellular fluid. Because sodium intake is usually high in Western society, potassium requirements correspondingly increase to maintain the balance (Syzdek and Jaroslaw, 2007). In addition to its effects on neural and muscular activity, potassium is important for maintaining bone health. Non significant decrease was observed in $GroupA_2$ compared with $GroupA_1$ and $GroupB_2$ compared with $GroupB_1$ in Potassium ion concentration. This is in contrast with the findings of Ikewuchi and Ikewuchi, (2012) in which administration of aqueous extract of rhizomes of *Sansevieria senegambica* significantly increased K^+ concentration in alloxan induced diabetic wistar rats. The non significant decrease observed due to the treatment(pretreatment and posttreatment) suggest that *Afzelia africana* based diet helps to ameliorate kidney injury.

Within the extracellular fluid, the major cation is sodium. It helps control blood pressure and fluid balance and it supports the work of nerves and muscles. When sodium levels in the blood are too low (hyponatremia), excess fluid enters the cells and swelling occurs (Estevez *et al*., 2008). Sodium plays a critical role in maintaining charge balances in cell membranes, usually operating outside cell walls. Non significant decrease was observed in $GroupA_2$ compared with $GroupA_1$ and $GroupB_2$ compared with $GroupB_1$ in Sodium ion concentration. This is in contrast with the findings of Stephen *et al*. (2014) in which administration of ethanolic seed extract of *Mucuna puriens* significantly decreased Na^+ concentration in alloxan induced male wistar rats. The kidney excrete or retains sodium via the action of aldosterone, antidiuretic hormone (ADH, or Vasopressin), atrial natriuretic peptide (ANP), and other hormones(Schwartz *et al*., 1957). The osmolarity of the body fluid is greatly determined by the plasma concentration of sodium ion which has a regulatory effect on the blood volume; hence low levels of sodium ion could suggest low blood volume and reduced blood pressure. Similarly, the observed non significant decrease in plasma sodium ion concentration is an indication of increased renal excretion of sodium ion.

CONCLUSION

The results of this study shows that *Afzelia africana* based diet possess anti-diabetic potential and did not produce adverse effects on the kidney. Therefore, it can be concluded that *Afzelia africana* based diet is very promising for developing standardized phytomedicine for Diabetes mellitus. Further study should be carried out to identify more bioactive components in *Afzelia africana* seed and their mode of action in lowering blood glucose level.

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