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NEffect of *Vernonia amygdalina* Del Leaf on Kidney Function of Diabetic Rats

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Abstract: This research assessed the hypoglycemic action of ethanolic extract of *Vernonia amygdalina* del and its impact on selected indices of kidney function in experimental diabetic rat models. Twenty-one Wistar rats (120-160 g) assigned to 3 groups of seven rats each were used. Groups 1 and 3, constituting the diabetic (DC) and normal controls (NC), respectively were both given placebo treatment, whereas group 2 was the test group of animals administered the extract (400 mg kg⁻¹ body weight) by gastric-intubation for 14 days. Results of analyses of serum electrolytes and biochemical indices showed: significant reductions ($p < 0.05$) in glucose, urea and sodium concentrations of the *V. amygdalina* ethanolic extract treated group (144.14 ± 25.83 , 81.60 ± 16.52 and 65.00 ± 6.24 , respectively) relative to their respective controls (247.25 ± 4.83 , 122.08 ± 10.60 and 116.62 ± 12.00). Serum chloride levels of the test group also reduced, whereas, that of potassium and creatinine were elevated with respect to their normal control values. However, these later changes were non-significant ($p > 0.05$). Histological changes in the kidney tissues such as necrosis of tubules, degeneration of cells of glomerular capsule and partial obliteration of glomerular tuft observed in diabetic animals were reversed in extract treated diabetic group. *Vernonia amygdalina* extract besides its hypoglycemic action, can protect against kidney impairments due to diabetes, but may induce dilutional hyponatraemia.

Key words: *Vernonia amygdalina* del, electrolytes, kidney function, diabetes mellitus, serum glucose, kidney histology

INTRODUCTION

Diabetes mellitus remains one of the age-long chronic diseases of the human race and its frontiers are expanding by the day. This is so real that in 2004, the World Health Organization identified it as an epidemic underway, since about 191 million persons world wide were afflicted in 2000 and a possible increase to 366 million by 2030 was projected (Wild *et al.*, 2004). World Health Organization reports that over 4.8 million annual deaths (9% of global total) are attributable to either diabetes or its complications.

Despite these alarming statistics, a blanket therapy yet does not exist at least for now. However, chemotherapeutic drugs have been in use since the accidental discovery of the hypoglycemic action of sulfonamides in 1945 (Robinson and Johnston, 1997). Life style measures and insulin injections have additionally, been used. The thrust of such management measures is to achieve an effective blood glucose control or utilization, with a view to delaying or averting the onset of complications. Yet the measures are not without limitations and side effects.

Consequently, herbal medicine, a new promising approach has emerged-the use of natural products and synthetic agents, alone or in combination to prevent diabetes in humans. Ervin *et al.* (1999) in their survey

have indicated that 40-60% of diabetics use non-conventional treatments and 23% of this number use botanicals alone. WHO (1989) in its General Assembly, authenticated this approach (Bailey, 1989; Dey *et al.*, 2002). The use of natural remedies for diabetes management is also strengthened due to the belief that herbs can provide some benefits over and above allopathic medicine and allows users to feel that they have some control in their choice of medication (Joshi and Kaul, 2001).

Be that as it may, it is only appropriate that these herbs receive a holistic scientific scrutiny. Besides their hypoglycemic action, such treatment options should impart biochemical parameters of plasma and organs occupied with biotransformation functions to the advantage of the patients, especially those indices whose regulation is strongly correlated to glucose homeostasis, with an overall aim of ameliorating or at most delay the onset of complications of the disease if not cure.

It is a known fact that kidney function is compromised in uncontrolled diabetes mellitus. Glycosuria, a cardinal and diagnostic feature of diabetes imposes dehydration via glucose osmotic diuresis. This dehydration is accompanied with severe loss of electrolytes including sodium, potassium, calcium, chloride and phosphates (Gaw *et al.*, 1995). Also in diabetes there is abnormally increased ketone body

formation leading to ketonuria. Ketone bodies being moderately strong acids, on excretion carried along side with them buffer cations particularly alkaline cations (Na^+ and K^+) and also bicarbonates (Ramsey, 1986). Additional, substances otherwise not present in urine are excreted in urine including albumin (microalbuminuria) in diabetic condition. This undue passage distorts the repellent ability of structural polysaccharides (e.g., hyaluronic acids) whose function is to maintain the integrity of the kidney cells (Gaw *et al.*, 1995), hence a distortion in the kidney basement membrane cell integrity. A combination of these factors and many more culminates in compromised kidney function in diabetes mellitus.

V. amygdalina del (commonly called bitter leaf in Nigeria), a shrub belonging to the plant family Asteraceae or Compositae and grows predominantly in a range of ecological zones in tropical Africa (Farombi, 2003) has received tremendous attention from researchers.

In an ethno botanical survey which identified and documented 22 plants of South Western Nigeria used by traditional healers in this region in the management of diabetes, *V. amygdalina* del is second only to *Cassia alata* as the most frequently used (Abo *et al.*, 2000).

In this study the possible changes in kidney function parameters associated with the use of *V. amygdalina* del in the management of diabetes and the histological integrity of the tissues were investigated, so as to relate this to its anti-hyperglycemic/hypoglycemic action.

MATERIALS AND METHODS

Collection and preparation of plant extract: Fresh matured leaves of *V. amygdalina* del were harvested from the Endocrine Research Farm of the University of Calabar, Calabar. These were rinsed with distilled water and dried under shade. The dried leaves were ground into powder after which 100 g of the coarse powder was suspended in 600 mL of ethyl alcohol (98.67%, BDH) and agitated thoroughly for about 10 min with an electric blender. The suspension was allowed overnight in a Westcool fridge (4°C).

Twenty four hours later, the mixture was filtered with a chess cloth and the filtrate concentrated *in vacuo* (rotary evaporator) to 10% (1/10) of the original volume at 40°C . This concentrate was allowed in a water bath at 37°C for complete dryness, yielding 35.0 g (35% yields) of the crude extract. This extract was reconstituted in distilled water to an appropriate concentration before administration.

Animals and animal grouping: Twenty-one rats (males and females) of Wistar strain weighing 120-160 g obtained

from the animal house, Department of Human Anatomy, University of Calabar were used for the study. They were allowed one week of acclimatization after which the animals were reweighed and housed in plastic cages with plastic bottom and wire mesh top (North Kent Co. Ltd), under controlled environmental conditions of temperature ($28\pm 2^\circ\text{C}$) and relative humidity ($50\pm 5\%$) and a 12 h light/dark cycle. The animal house facility was adequately ventilated and the animals maintained regularly on a commercial rat chow, which was together with tap water given *ad libitum*.

The design consisted of 3 groups of 7 rats each:

- Group I: The Diabetic Control (DC), received 0.2 mL distilled water
- Group II: The test group-diabetic treated group, received 400 mg kg^{-1} body weight of extract and
- Group III: Consisting of Normal Control (NC) also received 0.2 mL distilled water

Treatment was administered twice per day by gastric intubation in a 12 h cycle (7:00 am and 7:00 pm) everyday for 14 days.

Induction of experimental diabetes: Diabetes was induced by intra-peritoneal injection of 150 mg kg^{-1} body weight of alloxan monohydrate (Sigma, St. Louis, MO, USA) using distilled water as the vehicle. Five days later, diabetes was confirmed in the alloxan treated animals with Random Blood Glucose (RBG) level = 200 mg dL^{-1} (11.1 mmol L^{-1}). Diabetes status determination was performed using an automated glucose sensor machine-Glucometer Analyser (One-Touch, Basic).

Sample collection for analyses: Twelve h after last feeding and administration (overnight fast), the animals were euthanized under chloroform vapour and dissected. Whole blood obtained by cardiac puncture into non-heparinized tubes were allowed to clot for about 2 h and thereafter centrifuged ($4,000\text{ g}$ for 10 min) to remove cells and recover serum, which was used for the biochemical assays. The kidneys were surgically removed and decapsulated then cleansed of blood with 0.25M sucrose solution and thereafter fixed in 10% formaldehyde.

Biochemical assays

Serum glucose: Dialab kit method based on Barham and Trinder (1972) was used. The principle is based on glucose oxidase reaction, in which the enzyme oxidizes glucose to gluconic acid and hydrogen peroxide formed

as a byproduct. The peroxides whose concentration is in proportion to glucose in sample develops quantifiable colour via 4-aminophenazone in the presence of a peroxidase.

Potassium in serum: Potassium in serum was determined by photometric turbidimetric test (Tietz, 1976) of TECO analytical kits. Potassium ions in a protein-free alkaline medium react with sodium tetraphenylboron to produce a finely dispersed turbid suspension of potassium tetraphenylboron, whose turbidity is in proportion to the potassium concentration originally in the sample.

Sodium in serum: Serum sodium concentration was estimated, using Mg-Uranylacetate method of HUMAN diagnostic kits based on Trinder (1951). Sodium in serum is precipitated with Mg-uranylacetate, the remaining uranyl ions form a yellow-brown complex with thioglycolic acid. The difference between reagent blank and analysis is proportional to the sodium chloride.

Serum chloride: Chloride in serum was determined using mercuric thiocyanate method (Dialab Diagnostic Kits based on Tietz, 1976). Chloride ions in the sample react with mercuric thiocyanate displacing the thiocyanate ions. The displaced thiocyanate ions react with ferric ions producing a coloured complex.

Serum urea: Urea in serum was estimated by Endpoint colourimetric (Dialab Kit based on the principles of Searcy *et al.*, 1967). Urease enzyme hydrolysis urea to ammonia and carbon dioxide. The NH_3 so formed reacts with alkaline hypochloride and sodium salicylate in the presence of sodium nitroprusside to form a coloured chromophore.

Creatinine in serum: The assay is based on the reaction of creatinine with an alkaline solution of sodium picrate to form a red complex (Newman and Price, 1999). The red colour is proportional to the concentration of creatinine in the sample.

Histopathological study: The fixed kidney tissues were sectioned (5-micron thickness) and sections stained with Haematoxylin and Eosin (H&E) according to Conn (1946) procedure and Photomicrographs developed (x 400).

Statistical analysis: All data are expressed as mean \pm SD. Pair wise comparison between test and control groups were done using the student t-test. Differences between groups were considered significant at $p < 0.05$.

RESULTS

Changes in blood glucose level, serum sodium, potassium, chloride, urea and creatinine of alloxan induced diabetic rats following oral administration of ethanolic extracts of *V. amygdalina* del are shown in Table 1. Diabetic untreated animals showed significantly raised ($p < 0.05$) serum levels of glucose (247.25 ± 4.83), urea (122.08 ± 10.66) and creatinine (0.73 ± 0.04) compared to the respective normal controls (73.51 ± 18.98 , 57.27 ± 5.10 and 0.69 ± 0.03). In the diabetic rats treated with the extracts, the levels of these indices reduced significantly ($p < 0.05$) to 144.14 ± 25.83 , 81.60 ± 16.52 and 0.61 ± 0.06 respectively). Serum sodium and chloride decreased following diabetes induction compared to the normal controls. This decrease was however, significant ($p < 0.05$) only in sodium levels (from 150.88 ± 2.06 to 116.62 ± 12.00). Treatment with extract further decrease the sodium levels (65.00 ± 6.24) significantly ($p < 0.05$) and the chloride non-significantly ($p > 0.05$) compared to their diabetic controls. Changes in potassium levels were reciprocal to that of sodium except that the changes for sodium were non significant ($p > 0.05$).

Diabetic control kidneys (Fig. 1) showed that hyperglycemia caused patches of complete (Gc) and partial (Gp) obliteration of the tuft of glomerulus, degeneration of the cells of Glomerular Capsule (Gc) - widening of the Renal Space (RS) and necrosis of the tubules also observed (Tn). These changes were reversed in the extract treated kidneys (Fig. 2) where the glomerular tuft became regenerated (G); necrotic tubules appeared more convoluted, with regeneration of its endothelial cells

Table 1: Effect of ethanolic extract of *V. amygdalina* Del on serum glucose and some selected indices of kidney function of alloxan-induced diabetic wistar rats treated for 14 days

Group	Glucose (mg dL ⁻¹)	Potassium (K ⁺) (meq L ⁻¹)	Sodium (Na ⁺) (meq L ⁻¹)	Chloride (Cl ⁻) (mg dL ⁻¹)	Urea (mg dL ⁻¹)	Creatinine (mg dL ⁻¹)
Diabetic control (0.2 mL dist. Water)	247.25 \pm 4.83 **	4.99 \pm 0.54	116.62 \pm 12.00 **	97.10 \pm 2.14	122.08 \pm 10.66**	0.73 \pm 0.04
Diabetic treated (400 mg kg ⁻¹ of extract)	144.14 \pm 25.83**	4.40 \pm 0.36	65.00 \pm 6.24*	94.39 \pm 3.06	81.60 \pm 16.52*	0.61 \pm 0.06
Healthy control (0.2 mL dist. Water)	73.51 \pm 18.98	4.73 \pm 0.46	150.88 \pm 2.26	99.69 \pm 4.04	57.29 \pm 5.10	0.69 \pm 0.03

Values represent mean \pm SD, n = 7* = Statistically significant ($p < 0.05$) compared to diabetic control. ** = Statistically significant ($p < 0.05$) compared to normal control

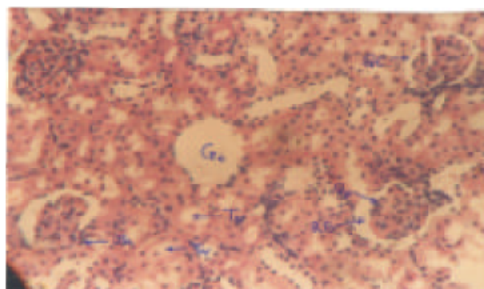


Fig. 1: Histomorphology of the kidney of the diabetic control group stained with H and E (x 400).
GC = Glomerular Capsule, G_c = Obliterated Glomerulus (complete) G_p = Obliterated Glomerulus (partial), T_n = Tubules Necrosis

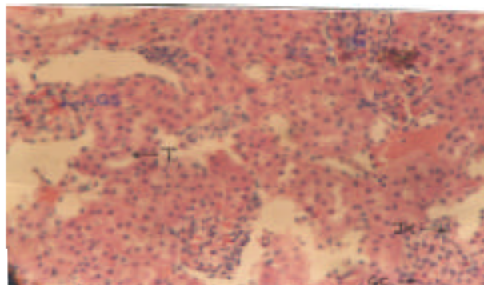


Fig. 2: Histomorphology of the kidney from diabetic treated group stained with H and E (x400).
G = Glomerulus, GS = Glomerulosclerosis, GC = Glomerular capsule, JX = Juxta Glomerular apparatus, RS = Renal space

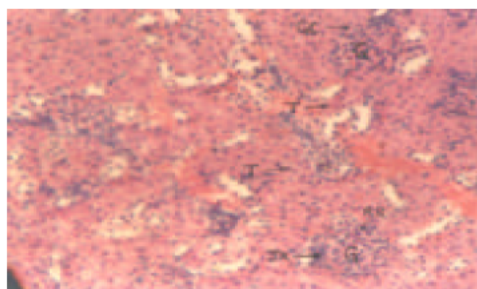


Fig. 3: Histomorphology kidney of normal control group stained with H and E (x 400). GC = Glomerular capsule, G = Glomerulus, T = Tubules, RS = Renal space, JX = Juxta Glomerular apparatus

in some (T); regenerated Glomerular Capsule (GC) as well as juxtaglomerular apparatus were stained properly, hence a recovery from the hyperglycaemia-induced lesions observed in Fig. 1. Normal kidney histology,

demonstrated well stained tuft of glomerulus, glomerular capsule close-association with the glomerulus, hence small urinary space (Fig. 3). Tubule cells and juxtaglomerular apparatus, distinct and well stained. Though the reversal in lesions of the treated group was not total, the integrity was not significantly different from the normal control (Fig. 3).

DISCUSSION

The result of this investigation indicates clearly that oral administration of ethanolic leaf-extract of *V. amygdalina* del substantially reduced blood glucose level in alloxan-induced diabetic rats. The result is consistent with earlier reports (Akah and Okafor, 1992; Nimenibo-Uadia, 2003; Gyang *et al.*, 2004; Uhegbu and Ogbuehi, 2004 and Nwanjo, 2005) on the hypoglycemic and antihyperglycemic action of extracts of *V. amygdalina* in rats. Nimenibo-Uadia (2003) attributed the role of this reaction to tannins present in extract of *V. amygdalina*, whereas Akah and Okafor (1992) and Akah *et al.* (2004) presupposed a mechanism unrelated to insulin secretion from pancreatic β -cells. Be that as it may, it is probable that two mechanisms may exist: One related to insulin production and the other targets peripheral carbohydrate metabolism. The former endows it the ability to exert hypoglycemia in diabetic condition (antihyperglycemia), whereas the later achieves hypoglycemia in non-diabetic rats. Alloxan is known to mediate pancreatic β -cell destruction via ROS generation (Szkudelski, 2001), depriving the animal of insulin, hence diabetes. *V. amygdalina* having the ability to abate this alloxan-induced diabetes must necessarily have a corrective impact on the hitherto destroyed β -cells of the pancreas. It's plausible to suppose that the powerful antioxidant effect of *V. amygdalina* reported by Igile *et al.* (1994) to include luteolin, 7-O-beta-glucuronoside and luelin, 7-O-betaglucosides may have attempted the reversal of the cytotoxic action of alloxan or at least mopped up the free radicals generated by alloxan responsible for beta cell destruction. By so doing the β -cells could have started a gradual regeneration, hence insulin production commencing to start an effectual control of hyperglycemia. To some extent this may proffer an explanation to why the reduction in serum glucose is not a total return to normoglycemia, as the β -cells may not have completely been regenerated. On the other hand, the phytochemicals endowed *V. amygdalina* may possess some alpha-glucosidase inhibitors as secondary plant metabolites. Such metabolites may competitively inhibit intestinal brush border enzymes-glucosidases, as well as pancreatic beta-amylase with the ultimate reduction in

digestion and subsequent absorption of carbohydrates from the gut, i.e. post prandial hyperglycemia. Nimenibo-Uadia proposed such a parallel mechanism for tannins in his discourse. This is strengthened by Winleman (1989), who indicated a strong positive correlation between the presence of flavoniods glycosides and phytosterols of plants with hypoglycemic and antihyperglycemic activities, respectively. It's probable that *V. amygdalina* may be endowed with a combination of these phytochemicals.

Serum sodium level was observed to decrease significantly following diabetes induction. Glucose excretion in urine by diabetics imposes an osmotic diuresis (dehydration) (Loeb, 1991) with the consequence of electrolyte lost with dehydration. Also, ketoacidosis is a prevalent feature which leads to ketonuria. An attempt by the kidney to buffer the urine decreases the alkaline metals including potassium and sodium in serum (Ramsey, 1986). Treatment with extract further decreased serum sodium levels. It is possible that as treatment gradually clears glucose from blood and urine, water lost (dehydration) also gradually disappears and ketoacidosis stimulus also decreases. This could lead to water retention in the ECF to cause dilutional hyponatraemia. The mechanism follows in tandem for chloride. The change in sodium is prominent probably because it is the most abundant metallic ion in the ECF (Werner, 1983). Changes in potassium levels in serum usually should alternate those of sodium (Loeb, 1991). Diabetic acidosis prominent in most diabetes usually decrease potassium excretion in urine, hence an increased retention in serum (Nduka, 1997). That the change in potassium is not significant may serve to differentiate the observed dilutional hyponatraemia from that due to increased sodium concentration in serum.

Urea level in diabetic control was significantly higher with respect to the normal animals. This observation is expected. Deficiency of insulin, an anabolic hormone and inability of glucose to reach the extra-hepatic tissues stimulates gluconeogenesis as an alternative route of glucose supply (Robinson and Johnston, 1997). Gluconeogenesis is sustained by increased proteolysis which releases free glucogenic amino acids circulated in plasma. These are deaminated in the liver with the consequence of increased urea in blood. As glucose is cleared from serum and insulin effect probably reintroduced, proteolysis reclines via hormonal stimulation (Robinson and Johnston, 1997), hence decreased urea concentration in blood. Observation of creatinine concentration parallels that of urea. Creatinine is a metabolite of muscle creatine, whose amount in serum is proportional to the body's muscle mass. The amount of creatinine is usually constant, so that elevated levels

indicate diminished renal function only, since it is easily excreted by the kidneys (Loeb, 1991). Diabetic kidneys as observed in this study, are prone to impairments. Extract treatment however, produced a reduction in creatinine levels supposing a protection against impairment due to diabetes.

The changes at the molecular level imparts on the gross architecture of the kidney tissues. A comparison of the plates (histopathology) of the kidney reveals that the degeneration of the glomerular capsule, necrosis of tubule cells and obliteration of glomerular tuft observed in untreated diabetic group were reversed following extract treatment. These pathologic changes were induced by the oxidative stress associated with diabetes. Jimoh and Odutaga (2004) had reported alterations and disintegration of the glomeruli of kidneys as a consequence of free radicals generated by thermoxidised lipids. It's likely that these pathological changes led to disruption in filtration and concentration of urine, as well as fluid and electrolyte balance, as is observed from biochemical assays. Administration of extract reversed the impressed factor responsible for oxidative stress-hyperglycemia, hence a corrective measure even on the histology of the kidney.

This study suggests therefore, that extract of *V. amygdalina* del besides its hypoglycemic/anti-hyperglycemic action could protect the kidneys against impairment due to diabetes, but may induce dilutional hyponatremia as a side effect.

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