

Effects of Co-administration of Extracts of *Vernonia Amygdalina* and *Azadirachta Indica* on Serum Electrolyte Profile of Diabetic and non Diabetic Rats.

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Abstract: This work assessed the changes in serum electrolyte profile following oral administration of leaf-extracts of *Vernonia amygdalina* (VA) and *Azadirachta indica* (AI) singly and in combination, and subcutaneous insulin to diabetic and non diabetic rats for 28 days. Serum concentrations of K, Na, Cl and P of diabetic control rats decreased relative to non diabetic control, although this decrease was significant ($P < 0.05$) in phosphorous (hypophosphataemia) and sodium (hyponatraemia) only. Treatment with VA extract alone further decreased significantly ($P < 0.05$) and non-significantly ($P > 0.05$) sodium and potassium concentrations respectively. However, these hyponatramic and hypokalaemic actions were effectively modulated, with administration of the combined extracts. There were no significant changes in serum calcium levels in all test groups as well as serum K, Na and Cl levels of the non-diabetic rats which received treatment. Serum phosphorus concentration which was significantly reduced ($P < 0.05$) in the diabetic control rats was also further decreased significantly in the test animals upon administration of VA extract alone relative to both controls. Unlike the case with sodium, co-administration with AI extracts could not modulate VA effect, rather it caused a steeper 4.94 and 3.20 fold decreases ($P < 0.05$) in serum phosphorus concentration relative to diabetic and non-diabetic controls respectively, and a 2.73 fold decrease in non-diabetic test group compared to non-diabetic control. Combined administration of VA and AI extracts can reverse dilutional hyponatraemia in diabetes complication and of management with VA extract but may amplify the diabetes induced hypophosphataemia.

Key words: *Vernonia amygdalina*, *Azadirachta indica*, Serum electrolyte, diabetes mellitus, hyponatraemia and hypophosphataemia

INTRODUCTION

Electrolyte imbalance secondary to compromise in kidney function in prolonged and uncontrolled hyperglycemia of diabetes mellitus has long been established. Usually, glycosuria, a prominent diagnostic feature of diabetes mellitus imposes dehydration via glucose osmotic diuresis, which is usually accompanied with severe loss of electrolytes including sodium, potassium, calcium, chlorine and phosphates (Gaw *et al.*, 1995; Ramsey, 1989). Also, due to increased ketone body formation from the so-called 'overflow' pathway of fat metabolism, diabetics are also known to produce acid urine. As a physiological response, buffer cations, particularly the alkaline cations (Na^+ and K^+) and bicarbonates are depleted via excretion, in an attempt to buffer the urine. There is, therefore, an over-exertion on volume control in diabetes mellitus which any effective or right treatment/management option must seek to address, besides its antihyperglycemic and/or hypoglycaemic action. Volume control is fundamental in the management of diabetes mellitus, since it would usually result to shocks, seizures or termination of life if not properly controlled.

Leads from traditional medicine and the multifaceted therapeutic agents of herbs, given the multiplicity of bioactive agents endowed in them has of recent aroused severe and intense research interest in antidiabetic medicinal plants, particularly when the herbs are used in combination – polyherbal therapy. *Vernonia amygdalina* (Del.) and *Azadirachta indica* (A. Juss) are plants traditionally used in the management of diabetes in the African Sub-region (Abo and Adediwora, 2000) and Asia (Biswas *et al.*, 2002) respectively. Their individual hypoglycaemic and antihyperglycemic actions have additionally been compared (Ebong *et al.*, 2006).

Recently in our laboratory, further investigations show that a combination of extracts from these two plants may be more efficient in their antidiabetic activity compared to their extracts (Ebong *et al.*, 2008). It became

imperative therefore to, besides the anti-glycaemic action, test the action of this combined extracts on associated imbalances of hypoglycaemia in diabetes. Therefore, this study investigated the effects of co-administration of extracts of VA and AI on the electrolyte profile of diabetic and non-diabetic rats with a view to ascertaining the over all efficacy of this potential therapeutic option.

MATERIALS AND METHODS

Collection of plant Materials:

Matured leaves of *Vernonia amygdalina* (Del.) and *Azadirachta indica* (A. Juss) were respectively collected from the Endocrine Research Farm, as well as the staff village, University of Calabar, in February 2008. They were authenticated by Dr. E. G. Amanke, a Plant Ecologist, Department of Botany, University of Calabar, Calabar and Voucher specimens deposited in a herbarium (ERU/2006/011 and ERU/2006/012 respectively) in the Department of Botany. The leaves were rinsed severally with clean tap water to remove dust particles and debris and thereafter allowed to completely drain.

Preparation of Plant Extracts:

The plant materials were separately cut and chopped into bits with a knife on a chopping board. One kilogram (1kg) each of *A. indica* and *V. amygdalina* was homogenized with an electric blender in 1.95 and 2.25 litres of 80% (v/v) ethanol respectively. The mixtures were allowed for 48hrs in the refrigerator at 4°C for thorough extraction of the plants' active components. These were then filtered with cheesecloth and later with Whatman No. 1 filter paper to obtain a homogenous filtrate. These filtrates were then concentrated *in vacuo* at low temperature (37- 40°C) to about one tenth the original volume using a rotary evaporator. The concentrates were allowed open in a water bath (40°C) for complete dryness yielding 40.54g (4.054%) and 34.71g (3.471%) of greenish brown and brown oily substances for *V. amygdalina* and *A. indica* respectively. The extracts were then refrigerated at 2- 8°C until use.

Animals and Experimental Design:

Sixty albino rats (males only) of Wistar strain weighing about 140-180g were obtained from the animal house of the Department of Zoology and Environmental Biology, University of Calabar, Calabar. The animals were allowed to acclimatize for three weeks in the animal house of the Department of Biochemistry. The animals were housed in well ventilated cages (wooden bottom and wire mesh top) and kept under controlled environmental conditions of temperature (25 ± 5°C), relative humidity (50 ± 5%) and 12 hour light / dark cycle. The 60 rats divided into 5 parallel groups consisting of a diabetic and non-diabetic pair of 6 animals each (table 1).

Induction of Experimental Diabetes:

Prior to diabetes induction, the rats were subjected to 12hr fast, and then diabetes was induced by intra-peritoneal injection of 65mg/kg b.w. with streptozotocin (STZ) (Sigma St. Louis, MO, U.S.A) ^[7] in our procedure reconstituted in normal saline. Control animals received saline only. Seven days after STZ treatment, diabetes was confirmed in STZ treated rats with a fasting blood sugar concentration ≥ 200mg/dl. This was estimated using One Touch[®] Glucometer (Lifescan, Inc. 1995 Milpas, California, U.S.A) with blood obtained from the tail vein of the rats.

Experimental Protocol:

Diabetic and non-diabetic animals were grouped as shown in table 1 and accordingly, treated with extracts and insulin. The dosages of the plant extracts were as determined from preliminary work in our laboratory whereas insulin dose, NPH (5U/kg b.w. s.c.) was as previously used by Sonia and Srinivasan (1999) and also chosen to simulate human regimen. The plant extracts were administered via gastric intubation, twice per day (6.00am: 6.00pm) and insulin once per day post prandial (6.00pm). The animals were maintained on palletised Growers Feed obtained from Vital Feeds, Jos, Plateau State, Nigeria, and tap water. Both the feed and water were provided *ad libitum* and the treatment lasted for 28 days.

Collection of Samples for Analysis:

At the end of the 28 days, food was withdrawn from the rats and they were fasted overnight but had free access to water. They were then euthanized under chloroform vapour and sacrificed. Whole blood was collected via cardiac puncture using sterile syringes and needles, emptied into plain tubes and allowed to clot for about

two hours. The clotted blood was thereafter centrifuged at 3,000rpm for 10 minutes to recover serum from clotted cells. Serum was separated with sterile syringes and needles and stored frozen until used for electrolyte profile analysis.

Estimation of Serum Electrolytes:

Chloride, calcium and phosphorus concentrations in serum were determined using biochemical assay kits obtained from DIALAB Produktion und laborinstruments Gessellschaft m.b.H. A-1160 Wien-panikengasse, Austria, according to the method of Burtis and Ashwood (1999), whereas sodium and potassium kits were obtained from TECO Diagnostics 1268 N. Lakeview Ave. Anaheim, CA 92807, U.S.A. and determinations according to the method described by Tietz (1976).

Statistical Analysis:

The results were analysed for statistical significance by one way ANOVA using the SPSS statistical program and Post Hoc Test (LSD) between groups using MS excel program. All data were expressed as Mean ± SEM. P values < 0.05 were considered significant.

RESULT AND DISCUSSION

Effect of Treatment on Serum Electrolyte Levels of the Experimental Animals:

Results of changes in selected serum electrolyte concentrations – potassium (K), sodium (Na), chloride (Cl), calcium (Ca) and phosphorus (P), after a 28-day treatment with extracts of VA, AI singly and combined, and insulin in both non diabetic and diabetic rats are respectively shown in tables 1 and 2. Serum concentrations of K, Na, Cl and P of control STZ diabetic rats were decreased compared to the non-diabetic control. However this decrease was only significant in phosphorus and sodium concentration. Treatment of the diabetic rats with extracts of VA alone, further decreased significantly (p < 0.05) serum sodium concentration. Potassium level was also significantly reduced compared to the non-diabetic control. This hyponatremic and hypokalemic action of VA extracts was effectively modulated in combined treatment with VA and AI, as K and Na concentrations in this group compared well with their respective non-diabetic control values. Chloride concentration in diabetic rats treated with insulin increased significantly (p < 0.05) relative to diabetic group treated with VA, but compared well with levels in the group treated with a combination of extracts. Changes in K, Na and Cl levels in non-diabetic group treated with extracts and insulin were all non-significant (p > 0.05). No significant change was observed in serum calcium levels in all test diabetic and non-diabetic groups. Serum phosphorus concentration which was significantly decreased in diabetic control rats was further decreased (p < 0.05) by treatment with extracts of VA, and this decrease was significant (p < 0.05) when compared to non-diabetic control. Although AI extract caused no significant change in phosphorus level, it could not modulate the effect of VA when given as combined extracts. A steeper 4.94 and 3.20 fold decreases (p < 0.05) were observed compared to the diabetic and non-diabetic controls respectively. Whereas, in the non-diabetic, treatment with individual extracts caused no significant change, a combination of extracts of VA and AI caused significant (2.73 fold) decrease in serum phosphorus level compared to non-diabetic control. Combined administration of VA and AI can reverse hyponatremia of diabetes complication and of VA treatment but not hypophosphatemia.

Table 1: Experimental design

Diabetic rats			Non-Diabetic rats		
Group	No. of animals	Treatment	Group	No. of animals	Treatment
1	6	Placebo (Diabetic control)	1	6	Placebo (Diabetic control)
2	6	<i>V. amygdalina</i> extract (200mg/kg b.w)	2	6	<i>V. amygdalina</i> extract (200mg/kg b.w)
3	6	<i>A. indica</i> extract (200mg/kg b.w)	3	6	<i>A. indica</i> extract (200mg/kg b.w)
4	6	<i>V. amygdalina</i> and <i>A. indica</i> combined extracts (100mg/kg b.w each)	4	6	<i>V. amygdalina</i> and <i>A. indica</i> combined extracts (100mg/kg b.w each)
5	6	Insulin (5 unit/kg b.w)	5	6	Insulin (5 unit/kg b.w)

Table 1: Effect of treatments on serum electrolytes profile of non diabetic rats.

Roup / Treatment	Potassium (mEq/L)	Sodium (mEq/L)	Chloride (mEq/L)	Calcium (mg/dl)	Phosphorus (mg/dl)
NC	6.39±0.38	151.87±11.71 ^a	108.08±2.44	9.73±0.33	8.01±0.91 ^a
VA _{ND}	5.72±0.28	133.74±6.79	110.30±2.93	10.82±0.33	7.41±1.53 ^c
AI _{ND}	5.59±0.26	142.98±8.33	108.84±1.89	11.60±1.36	6.53±1.03 ^c
VA/AI _{ND}	5.47±0.28	135.81±4.26	113.83±1.86	11.87±0.67	2.93±0.36 ^{a,d,b}
HU _{ND}	6.03±0.42	137.93±9.16	108.00±2.67	11.12±0.65	6.70±0.56

Mean \pm SE, n = 6, ND = non diabetic, HU = insulin, NC = non diabetic control
 a = p < 0.05 vs DC, b = p < 0.05 vs NC, C = p < 0.05 vs VA/AI

Table 2: Effect of treatments on serum electrolytes profile of streptozotocin diabetic rats.

Group / Treatment	Potassium (mEq/L)	Sodium (mEq/L)	Chloride (mEq/L)	Calcium (mg/dl)	Phosphorus (mg/dl)
NC	6.39 \pm 0.38	151.87 \pm 11.71 ^a	108.08 \pm 2.44	9.73 \pm 0.33	8.01 \pm 0.91 ^a
DC	5.24 \pm 0.55	120.58 \pm 12.70	106.84 \pm 4.46	11.05 \pm 0.08	5.18 \pm 0.89
VA _D	4.91 \pm 0.55 ^b	101.29 \pm 12.97 ^{b,a,c}	93.90 \pm 11.90	12.22 \pm 0.91	3.98 \pm 1.08 ^{c,b}
AI _D	4.84 \pm 0.22	132.64 \pm 17.84	108.53 \pm 5.39	12.04 \pm 1.08	5.20 \pm 0.16 ^c
VA/AI _D	5.90 \pm 0.54	133.15 \pm 10.20	104.84 \pm 3.54	13.23 \pm 1.65	1.62 \pm 0.32 ^{a,b}
HU _D	5.28 \pm 0.29	109.65 \pm 11.29	112.89 \pm 3.48*	10.75 \pm 1.05	3.13 \pm 0.85

Mean \pm SE, n = 6, D = diabetic, NC = non diabetic control, HU = insulin, DC = diabetic control
 a = p < 0.05 vs DC, b = p < 0.05 vs NC, * = p < 0.05 vs VA, C = p < 0.05 vs VA/AI

Discussion:

Diabetes is characterised by increased volume and metabolites excretions via the kidneys, usually in excess of normal thresholds. These usually give rise to derangements in homeostatic balance with respect to electrolytes. In this study, serum sodium and phosphorus concentrations decreased significantly, while potassium and chloride were non significantly decreased in diabetic control rats. Treatment with extracts of VA caused a further significant decrease. These effects of diabetes induction and VA extract on sodium concentration in serum, agrees with our earlier report (Atangwho *et al.*, 2007b) where the authors attributed decrease in untreated diabetic state to dehydration and loss of cations to buffering of metabolic acidosis; and that with VA treatment, to dilutional hyponatremia. The non significant decrease in potassium levels with VA treatment was also indicated and explained by these authors. However, this decrease was not observed with extract of AI, and when combined extracts was administered to diabetic rats, the hyponatremia became reversed or non existent comparable to insulin treatment. A striking positive complementary action. The hyponatremic action of VA extract was suppressed effectively with combined extract treatment. This presents a good potential in diabetic management, because derangement in electrolyte is what usually leads to hypovolumic shock via depressed CNS leading to death in uncontrolled diabetes (Ramsey, 1986; Attah, 2000). Changes in chloride followed similar trend with sodium although these were non significant. This can be explained to the fact that sodium is most predominant in serum, so even at short term the effect can be so pronounced.

Marked changes were observed in serum phosphorus levels. The decreased serum concentration in untreated diabetic rats, is again related to concomitant loss together with other electrolytes incidental to dehydration (Gaw *et al.*, 1995; Ramsey, 1986). A further non significant decrease was also observed with VA extract treatment, but not AI. When treated with combined extracts of VA and AI, significant decrease in phosphorus concentration both in treated diabetic and non-diabetic rats was observed as opposed to the result of sodium concentration – hypophosphatemia. Phosphorus is predominantly an intracellular electrolyte, and like potassium, it enters cells from the extracellular fluid if the rate of glucose metabolism and utilization in the cell is increased^[13]. For instance in the treatment of diabetic coma with insulin, where glucose infusion is given alongside, plasma hypophosphatemia usually occurs (Crook, 2006) since tissue metabolism of glucose is increased as well, following the insulin action. It is possible that the extracts caused clearance of glucose from blood to tissue. This probably may have induced a concomitant clearance mechanism for phosphorus from plasma to tissue since inorganic phosphorus is needed in metabolism of glucose in the tissues (glycolysis and oxidative phosphorylation), hence the observed decrease of phosphorus concentration in serum. Moreover, our previous report has indicated insulin mimetic action of a combination of these extracts in diabetic and non diabetic rats (Atangwho *et al.*, 2009) Again, a corroboration of this argument is the fact that administration of exogenous insulin to enhance glucose uptake from blood of diabetic animals caused a 1.66 fold decrease in serum phosphorus, but not in the non-diabetic rats where insulin administration is not vital for glucose metabolism in the tissue. Decrease in phosphorus level in serum in combined extract treated rats can therefore be seen as another positive synergistic effect not observed in single extract treatments. However, care should be taken in choosing a more reduced and appropriate dose, since significant decrease in phosphorus levels was observed also in non-diabetic animals treated with combined extracts of VA and AI. The need for nutritional phosphate supplementation during management of diabetes with these leaves may also be advocated.

Dilutional hyponatremia and hypophosphatemia which are features of diabetic complications, with the former amplified by VA treatment but not by AI treatment, has been observed in this study. Combined administration of VA and AI reversed the dilutional hyponatremia but sustained hypophosphatemia. Hence, further endorsing the use of these extracts as a combination rather than single extracts in the management of

diabetes mellitus.

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