Vol. 27 (no. 2) 56–62 30 June 2015

Biokemistri

An International Journal of the Nigerian Society for Experimental Biology

Research Article

Implications of oral administration of extracts of *Acalypha wilkesiana* leaves on serum electrolytes, urea and creatinine in normal experimental rabbits

Kingsley Omage[1], Marshall A. Azeke [2] and Jerry N.E. Orhue[3]

[1]Department of Biochemistry, College of Basic Medical Sciences, Igbinedion University, Okada, Edo State, Nigeria.

[2]Department of Biochemistry, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma, Edo State. Nigeria.

[3]Department of Biochemistry, Faculty of Life Sciences, University of Benin, Edo State, Nigeria.

*Correspondence: Kingsley Omage: omagekingsley@yahoo.com; +2348030495102

ABSTRACT: The leaves of *Acalypha wilkesiana* are eaten as vegetables as part of the traditional management of hypertension in Nigeria. This study was therefore conducted to evaluate the implications of oral administration of extracts of *Acalypha wilkesiana* leaves, on serum electrolytes, urea and creatinine, in normal experimental rabbits. A total of eighteen (18) rabbits were randomized into three groups (A, B and C) of six animals each and treated with aqueous (A) and ethanol (B) extracts of *Acalypha wilkesiana* leaves. The extracts were administered orally at a dose of 300mg/kg body weight for a period of twenty-one (21) days. Group C animals served as control. Administration of the aqueous or ethanol extract, at a dose of 300 mg/kg body weight, to normal rabbits resulted in a significantly ($P < 0.05$) lower serum creatinine. Treatment with the aqueous or ethanol extract also resulted in a non-significantly ($P > 0.05$) lower serum urea, chloride, sodium and potassium, as compared with the control, in normal rabbits. Also, treatment with the aqueous extract resulted in a significantly ($P < 0.05$) higher, while administration of the ethanol extract resulted in lower ($P > 0.05$) serum calcium levels of the normal rabbits, as compared with the control.

KEYWORDS: Acalypha wilkesiana, Ethanol extract, Aqueous extract, Electrolytes, Urea, Normal Rabbits.

Received: 5 January 2015: Revised 10 April 2015; Approved: 12 April 2015.

INTRODUCTION

The set-point for hydrogen ion regulation is dependent on three known factors: the partial pressure of carbon dioxide (P_{CO2}) (Madias *et al*., 1979), the diets net acid load (Frassetto *et al*., 1996; Maurer *et al*., 2003), and the age-related decline in renal function (Frassetto *et al*., 1996; Frassetto and Sebastian, 1996). However, the body imperfectly responds homeostatically to perturbations of systemic acid-base equilibrium caused by variations in those factors. In fact, several studies have demonstrated that typical Western diets are net acid-producing and induce a low-grade metabolic acidosis of severity proportional to the diet net acid load as indexed by the steady-state renal net acid excretion rate (NAE) (Frassetto *et al*., 1996; Maurer *et al*., 2003) and increase in extracellular fluid volume.

The regulation of extracellular fluid volume is an important physiologic function of the body. Persistent increase in blood pressure, as a consequence of increase in extracellular fluid volume, is one of the risk factors for strokes; heart attacks, heart failure and arterial aneurysm, and is a leading cause of chronic renal failure (Pierdomenico *et al.,* 2009). Even a moderate elevation of arterial blood pressure can lead to shortened life expectancy. At severely high pressure, defined as mean arterial pressure 50% or more above average, a person can expect to live no more than a few years unless appropriately treated (Guyton and Hall, 2006).

Acalypha wilkesiana is frequently used in traditional medicine, exclusively or as a major constituent of many herbal preparations for the management or treatment of hypertension or increase in arterial blood pressure. The plant is native to the south pacific islands (Bismarck Islands, Fiji, Vanuatu). It is a plant of great ornamental value due to its showily colored foliage, widely cultivated in the tropical and subtropical countries. It belongs to the family Euphorbiaceae. The medicinal values of this plant lie in some chemical substances that produce a definite physiological action on the human body (Ekhaise *et al*, 2010; Jeruto *et al*, 2011; Omage *et al*, 2013). The most important of these bioactive constituents are alkaloids, tannins, flavonoids, steroids, Cardiac glycosides, terpenoids, anthraquinones, phytates and phenolic compounds (Dabai *et al*, 2013; Muhammad *et al,* 2013; Omage and Azeke, 2014). However, there is dearth of information about the scientific rational for its use in the management of increased blood pressure. Thus, the aim of this study is to determine the effects of this plant on serum electrolytes, urea and creatinine of normal experimental rabbits, with a view to evaluating its homeostatic implication.

.

MATERIALS AND METHODS

Plant Materials: Fresh *Acalypha wilkesiana* leaves were obtained from local gardens within Benin City and authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Benin City. The leaves were properly washed, air-dried and ground into fine powder.

Preparation of Ethanol Extract: Portion (100g) of the powdered leaves was soaked in 400ml of ethanol (95%) for 72 hours (3 days), with occasional stirring using a magnetic stirrer to ensure proper mixture of the vessel content. The content was then filtered using a sintered funnel, (which is equivalent to four folds of bandage or sheet of cheese cloth). The extract (filtrate) was then concentrated using rotary evaporator and weighed.

Preparation of Aqueous Extract: Portion (100g) of the powdered leaves was soaked in 400ml of distilled water for 72 hours (3 days), and treated as described above.

Experimental Animals: Eighteen (18) adult rabbits of the New Zealand strain, weighing between 0.9 – 1.5kg, purchased from local dealers at Aduwawa Cattle Market Benin City, were used for the study. The animals were kept in the animal house of the Department of Biochemistry, University of Benin and maintained on a 12-hour light and dark cycle in clean disinfected cages. They were allowed free access to feed (standard pelletized growers feed from UAC-Vital Feed, Jos, Plateau State) and water throughout the duration of the experiment. The animals were treated according to the International guidelines for the care and use of laboratory animals and allowed to acclimatize to the new environment for a period of three (3) weeks. They were then randomized into three (3) groups (groups A to C) of six (6) rabbits each.

Experimental Design: The animals in the different groups were treated as follows: Group A: Treated with Aqueous Extract; Group B: Treated with Ethanol Extract; Group C: Non-treated (Control)

Administration of Extracts: Five grammes of the dry mass (concentrated extracts) were suspended in distilled water for administration to the experimental animals. The extracts (aqueous or ethanol) were administered orally at a dose of 300mg/kg body weight for a period of twenty-one (21) days.

Collection of Blood Samples: Prior to treatment (Basal/day 0) with the extract, blood samples were collected from the veins located on the dorsal side of the ear lobes of the experimental animals (rabbits), using sterilized hypodermic needles. At days 7, 14 and 21 after treatment with the extracts, blood samples were also collected. Samples were collected in fluoride oxalate and plane (universal) bottles immersed in ice. Immediately after collection of blood, the tubes were centrifuged at 3,500 rpm for 10 minutes to obtain clear plasma (fluoride oxalate) and serum (plane bottle) for further analysis.

Assay Methods: Potassium and sodium was determined by the method of Tietz (1987), using flame photometer. The principle involves the evaporation of solutions of Potassium or sodium, when finely aspirated into a burner. The flame evaporated the solution leaving solid salts which dissociated to give neutral ground state atoms. Some of these atoms

were excited in the flame thus moving into a higher energy state. When these excited atoms fell back to the ground state, light of characteristics wavelength was emitted (590nm for Na^+ 970nm for K^+). The light then passed through a suitable filter onto a photosensitive element and the amount of the current produced was proportional to the amount of Na or K originally present in the sample. Chloride was determined by the method of Skeggs and Hoschstrasser (1964), where chloride ions formed a soluble, non-ionized compound, with mercuric ions and displaced thiocyanate ions from non-ionized mercuric thiocyanate. The released thiocyanate ions reacted with ferric ions to form a color complex that absorbed light at 480nm. The intensity of the colour produced was directly proportional to the chloride concentration. The method of Connerty and Biggs (1966) was employed for the determination of calcium. The method was based on the specific binding of o-cresolftalein complexone (OCC), a metallochromic indicator, and calcium at alkaline pH with the resulting shift in the absorption wavelength of the complex. The intensity of the chromophore formed was proportional to the concentration of total calcium in the sample. Urea was by Weatherbum (1967) (Urease Berthelot method). Urea in serum was hydrolyzed to ammonia in the presence of urease. The ammonia was then measured photometrically by Berthelot reaction at 546nm. Creatinine was determined by the method of Bartels and Bohmer (1972). The principle involved the reaction of creatinine in alkaline solution with picric acid to form a coloured complex. The amount of the complex formed was directly proportional to the creatinine concentration. The intensity of the red coloured complex was measured at 492nm.

Statistical Analysis: Data are represented as Mean ± S.E.M (n = 6). Significance of difference was tested by Student t-Test, ANOVA and Turkey-Kramer test, using the GraphPad Instat Version 3 (GraphPad Software Inc. San Diego, California U.S.A.). Statistical Significance was set at $P < 0.05$.

RESULTS

The effects of oral administration of extracts (aqueous or ethanol) of *Acalypha wilkesiana* leaves on serum electrolytes, urea and creatinine in normal experimental rabbits, are as described below.

The effects of extracts (aqueous or ethanol) of *Acalypha wilkesiana* leaves on serum sodium levels in normal experimental rabbits are as indicated in Table 1. At day 7 of treatment, the aqueous extract caused a significantly ($P < 0.05$) lower serum sodium levels in group A, while in group B (given ethanol extract) the extract caused a significantly ($P < 0.05$) higher serum sodium levels, as compared with group C (control). At day 14 of extract administration, the aqueous extract caused non-significantly ($P > 0.05$) higher sodium levels in group A, while in group B the ethanol extract caused a non-significantly ($P > 0.05$) lower serum sodium levels, as compared with group C. At day 21 of extract administration, in group A, the aqueous extract caused a non-significantly ($P > 0.05$) lower serum sodium levels, while in group B, the ethanol extract did not cause any significant difference ($P > 0.05$) in serum sodium levels, as compared with group C.

Serum potassium levels were also affected after extract administration, as indicated in Table 2. At day 7 of treatment, in group A, the aqueous extract resulted in a non-significantly ($P > 0.05$) lower potassium levels, while in group B, the ethanol extract resulted in a non-significantly ($P > 0.05$) higher serum potassium levels, as compared to group C. At day 14, administration of the aqueous extract to group A resulted in non-significantly ($P > 0.05$) higher serum potassium levels, while administration of the ethanol extract to group B resulted in significantly ($P < 0.05$) higher serum potassium levels, as compared with group C. Still, at day 21 of extract administration, the aqueous extract caused a non-significantly ($P > 0.05$) higher, while the ethanol extract caused a significantly ($P < 0.05$) higher serum potassium levels, as compared with group C.

Table 1: Serum sodium (mEq/L) of normal rabbits treated with aqueous (A) and ethanol (B) extracts of *Acalypha wilkesiana* leaves with control (C).

Serum Sodium (mEq/L)	Group A (Aq. Ext)	Group B (Et. Ext)	Group C (Control)
DAY 0	114.00 ± 2.31^{xa}	118.00 ± 2.08^{xa}	109.33 ± 4.91^{xa}
DAY 7	105.33 ± 1.67^{xb}	118.33 ± 3.33^{ya}	112.00 ± 2.00^{za}
DAY 14	111.00 ± 3.51^{xa}	106.33 ± 5.36^{xb}	107.67 ± 6.77^{xa}
DAY 21	97.67 ± 3.48^{xb}	102.33 ± 1.33^{xb}	102.33 ± 0.33^{xa}

Data represent Means ± S.E.M (n = 6). Means with different letter $^{a, b, c,}$ superscripts, along column, are significantly different ($p < 0.05$). Means with different letter $^{x, y}$ superscripts, along row, are significantly different ($p < 0.05$).

Table 2: Serum potassium (mEq/L) of normal rabbits treated with aqueous (A) and ethanol (B) extracts of *Acalypha wilkesiana* leaves with control (C).

Serum Potassium (mEq/L)	Group A (Aq. Ext)	Group B (Et. Ext)	Group C (Control)
DAY 0	4.43 ± 0.59^{xa}	4.93 ± 0.55^{xa}	5.17 ± 0.27^{xa}
DAY 7	4.53 ± 0.48^{xa}	5.00 ± 0.15^{xa}	4.83 ± 0.34^{xa}
DAY 14	5.07 ± 0.09^{xa}	7.33 ± 0.17^{yb}	4.87 ± 0.23^{xa}
DAY 21	5.70 ± 0.40^{xa}	12.33 ± 2.30^{yb}	5.27 ± 1.03^{xa}

Data represent Means ± S.E.M (n = 6). Means with different letter $^{a, b, c,}$ superscripts, along column, are significantly different ($p < 0.05$). Means with different letter $^{x, y}$ superscripts, along row, are significantly different ($p < 0.05$).

Table 3: Serum chloride (mEq/L) of normal rabbits treated with aqueous (A) and ethanol (B) extracts of *Acalypha wilkesiana* leaves with control (C).

Serum Chloride (mEq/L)	Group A (Aq. Ext)	Group B (Et. Ext)	Group C (Control)
DAY 0	130.21 ± 4.97^{xa}	122.92 ± 9.12^{xa}	102.60 ± 21.66^{xa}
DAY 7	94.27 ± 1.83^{xb}	86.98 ± 0.72^{xb}	82.94 ± 5.59^{xa}
DAY 14	84.44 ± 3.43^{xb}	83.93 ± 8.08^{xb}	94.53 ± 9.28^{xa}
DAY 21	84.27 ± 2.76^{xb}	68.67 ± 5.32^{yc}	84.93 ± 1.14^{xa}

Data represent Means ± S.E.M (n = 6). Means with different letter $^{a, b, c,}$ superscripts, along column, are significantly different ($p < 0.05$). Means with different letter $^{x, y}$ superscripts, along row, are significantly different ($p < 0.05$).

Table 4: Serum calcium (mg/dl) of normal rabbits treated with aqueous (A) and ethanol (B) extracts of *Acalypha wilkesiana* leaves with control (C).

Serum Calcium (mg/dl)	Group A (Aq. Ext)	Group B (Et. Ext)	Group C (Control)
DAY 0	10.75 ± 0.07^{xa}	10.58 ± 0.08^{xa}	10.81 ± 0.10^{xa}
DAY 7	9.17 ± 0.36^{xa}	9.80 ± 0.23^{xa}	10.49 ± 0.70^{xa}
DAY 14	10.07 ± 0.20^{xa}	9.62 ± 0.32^{xa}	10.49 ± 0.70^{xa}
DAY 21	12.52 ± 0.23^{xb}	11.48 ± 0.62^{ya}	11.46 ± 0.28^{ya}

Data represent Means ± S.E.M (n = 6). Means with different letter $^{a, b, c,}$ superscripts, along column, are significantly different ($p < 0.05$). Means with different letter $^{x, y}$ superscripts, along row, are significantly different ($p < 0.05$).

Thus, administration of the extracts (aqueous or ethanol) resulted in steady increases in serum potassium levels all through the period of treatment.

In Table 3, administration of the extracts (groups A & B) resulted in non-significantly (P > 0.05) higher chloride levels, as compared with group C, at day 7 of treatment. At day 14, treatment with the extracts (both test groups), caused chloride values that are non-significantly (P > 0.05) lower than that of the control group. After 21 days of treatment, there was no significant difference (P > 0.05) in group A, while in group B there was a significantly (P < 0.05) lower serum chloride levels, as compared with group C. Thus, administration of the extracts resulted in steady decreases in serum chloride levels

In Table 4, at day 7 & 14 after administration of extracts (aqueous or ethanol) of *Acalypha wilkesiana* leaf, at a dose of 300 mg/kg body weight, the extracts (A & B) caused non-significantly (P > 0.05) lower levels of serum calcium, as compared with the control group (C). But at day 21 of treatment, in group A (given aqueous extract), the extract resulted in a significantly (P < 0.05) higher serum calcium levels, as compared with group C. While between group B and group C, there was no significant difference (P > 0.05) in serum calcium levels.

At day 7, serum urea levels showed no significant difference (P > 0.05) in the test groups as compared with the control (Table 5).

Table 5: Serum urea (mg/dl) of normal rabbits treated with aqueous (A) and ethanol (B) extracts of *Acalypha wilkesiana* leaves with control (C).

Serum Urea (mg/dl)	Group A (Aq. Ext)	Group B (Et. Ext)	Group C (Control)
DAY 0	32.72 ± 2.73^{xa}	33.52 ± 2.62^{xa}	35.76 ± 2.76^{xa}
DAY 7	42.58 ± 1.46^{xa}	41.12 ± 2.86^{xa}	41.78 ± 0.88^{xa}
DAY 14	37.45 ± 1.23^{xa}	42.90 ± 4.44^{xa}	37.15 ± 5.00^{xa}
DAY 21	40.97 ± 7.23^{xa}	45.54 ± 5.79^{xa}	54.26 ± 0.84^{xa}

Data represent Means ± S.E.M (n = 6). Means with different letter $^{a, b, c,}$ superscripts, along column, are significantly different (p < 0.05). Means with different letter $^{x, y}$ superscripts, along row, are significantly different (p < 0.05).

Table 6: Serum creatinine (mg/dl) of normal rabbits treated with aqueous (A) and ethanol (B) extracts of *Acalypha wilkesiana* leaves with control (C).

Serum Creatinine (mg/dl)	Group A (Aq. Ext)	Group B (Et. Ext)	Group C (Control)
DAY 0	1.122 ± 0.07^{xa}	1.067 ± 0.09^{xa}	1.094 ± 0.03^{xa}
DAY 7	0.766 ± 0.08^{xb}	0.701 ± 0.04^{xb}	1.116 ± 0.08^{ya}
DAY 14	0.949 ± 0.08^{xa}	0.803 ± 0.15^{xa}	1.045 ± 0.02^{xa}
DAY 21	1.015 ± 0.03^{xa}	0.865 ± 0.03^{xa}	1.104 ± 0.12^{xa}

Data represent Means ± S.E.M (n = 6). Means with different letter $^{a, b, c,}$ superscripts, along column, are significantly different (p < 0.05). Means with different letter $^{x, y}$ superscripts, along row, are significantly different (p < 0.05).

At day 14, there was no significant difference (P > 0.05) between groups A & C, while in group B, the ethanol extract caused a non-significantly (P > 0.05) higher urea levels as compared with group C. At day 21, both extracts caused non-significantly (P > 0.05) lower urea levels as compared with the control. Thus treatment with the extracts (aqueous or ethanol) did not result in any significant effect on serum urea level, all through the treatment period.

In Table 6, at day 7, the administration of *Acalypha wilkesiana* leaf extracts in group A (treated with aqueous extract) and group B (given ethanol extract) caused significantly (P < 0.05) lower serum creatinine levels, as compared to group C (control). But at days 14 & 21, administration of the extracts to both test groups caused non-significantly (P > 0.05) lower serum creatinine, as compared with the control. Treatment with the extracts resulted in significant (p < 0.05) decreases at day 7 and non-significant (p > 0.05) increases at days 14 & 21 in both groups (A & B).

DISCUSSION

Electrolytes perform many important functions, including regulation of the amount of fluid (water) in the body and maintaining the blood pH within normal range. They also facilitate the passage of fluid between and within cells through osmosis and play a part in regulating the function of the neuromuscular, endocrine, and excretory systems. Electrolyte disorder is an imbalance of certain ionized salts (i.e. bicarbonate, calcium, chloride, magnesium, phosphate, potassium, and sodium) in the blood.

The effects of extracts (aqueous or ethanol) of *Acalypha wilkesiana* leaves on serum sodium levels in normal experimental rabbits are as indicated in Table 1. The decrease effect in the serum sodium levels of the test animals, as a result of the administration of the extracts, may be beneficial in the prevention of metabolic acidosis due to excess sodium, accompanied with chloride. This may also be beneficial in the maintenance of homeostasis or regulation of arterial blood volume that is important in hypertensive conditions, as it is associated with increased sodium levels.

Serum potassium levels were also affected after extract administration, as indicated in Table 2. Thus, administration of the extracts (aqueous or ethanol) resulted in steady increases in serum potassium levels all through the period of treatment. The ethanol extract is particularly more effective, comparatively. This may be due to the higher amount of potassium present in the ethanol extract as compared with that in the aqueous extract (Omage *et al*, 2013). Potassium is a main component of cellular fluid which helps in the regulation of neuromuscular function and osmotic pressure. By reducing extracellular volume and blood volume, the natriuretic effect of potassium is generally considered to be an important component of its antihypertensive effect. Thus, the increasing effect of the plant on serum potassium levels may be beneficial in the maintenance of homeostasis.

Administration of the extracts resulted in steady decreases in serum chloride levels. It is obvious that the extracts caused a reduction in the serum chloride levels of the experimental animals, with that of the ethanol extract being more significant. Serum chloride is an anion that regulates blood pressure, fluid and electrolyte balance, gastric fluid, and chloride shift in HCO_3^- transport in erythrocytes. Severe depletion of serum chloride levels (Hypochloremia) causes metabolic alkalosis characterized by mental confusion, slowed breathing, paralysis, muscle tension or spasm. While severe dehydration, kidney failure, hemodialysis, traumatic brain injury, and aldosteronism can cause hyperchloremia. The plant tends to be protective against these as the chloride levels of the test groups were significantly lower than that of the control. Care may however be taken as prolong use of the plant may lead to metabolic alkalosis due to Hypochloremia.

Abnormalities in serum calcium concentration may have profound effects on neurological, gastrointestinal, and renal function. Maintenance of the normal serum calcium is a result of tightly regulated ion transport by the kidney, intestinal tract, and bone, mediated by calcaemic hormones especially parathyroid hormone and 1, 25-dihydroxyvitamin D_3. As observed, the significant effect of the plant extracts on serum calcium levels of the experimental animals is the increase resulting from the aqueous extract after 21 days of treatment. Calcium may drop too low in those with cirrhosis because vitamin D levels become too low for proper calcium use. Alternatively, when cirrhosis leads to hepatorenal syndrome in which both the kidneys and the liver fail, the blood calcium can become elevated. This may be a possible cause of the increase in serum calcium after prolonged (21 days) administration of the aqueous extract. A variety of common disorders are responsible for abnormalities in the serum calcium. Treatment of both hypercalcaemia and hypocalcaemia is dependent on the underlying disorder, the magnitude of the deviation of the serum calcium, and the severity of symptoms.

Treatment with the extracts (aqueous or ethanol) did not result in any significant effect on serum urea level, all through the treatment period. Urea production occurs in the liver; it is found dissolved in blood and is excreted by the kidney as a component of urine. In most mammals, ability to concentrate urea in the urine exceeds that for electrolytes. The result showed that urea levels were either not significantly affected or not increased by the plant extracts. Thus, it helps in reducing the amount of waste nitrogen, as observed in the experimental animals. The handling of urea by the kidneys is a vital part of human metabolism. Besides its role as carrier of waste nitrogen, urea also plays a role in the countercurrent exchange system of the nephron that allows for re-absorption of water and critical ions from the excreted urine.

Creatinine is a waste product of muscle cell metabolism that is excreted by the kidneys in the urine; it is usually measured to check kidney function, with elevations indicating kidney problems. The decreases in creatinine levels, as observed

are indications of the normal functioning of the kidney, since the creatinine (a waste product of normal muscle metabolism) formed is being excreted. Thus, the plant seems not to affect the normal functioning of the kidney. Patients with chronic renal failure commonly present with muscle weakness and display disturbances in muscular Creatinine metabolism (Brautbar, 1983; Ogimoto *et al*, 1992).

Thus, administration of the extracts (aqueous or ethanol) caused a reduction in serum chloride and sodium levels and an increasing effect on serum potassium levels of the test animals. After administration, the aqueous extract resulted in a significant increase, while the ethanol extract resulted in a non-significant decrease in serum calcium levels of the test animals. The levels of serum urea were also non-significantly reduced, while serum creatinine levels were significantly reduced in the test animals. These show a positive effect of the plant with respect to maintenance of homeostasis.

Conclusion

The effects of *Acalypha wilkesiana* leaf extracts in causing a reduction in sodium, chloride, urea and creatinine levels, as well as in leading to increase in the level of potassium indicate that the plant has the potential to be used in maintaining homeostasis. These portend the plant as a possible remedy for the management of increased arterial blood pressure.

REFERENCES

Bartels H. and Bohmer M. (1972). Kinetic determination of creatinine concentration. Clin. Chem. Acta 37: 193–197.

Brautbar N. (1983). Skeletal myopathy in uremia: abnormal energy metabolism. Kidney International 24 Suppl 16: S81–S86.

Connerty H.V. and Biggs A.R. (1966). Determination of serum calcium by means of ortho-cresolphthalein complexone American Journal of Clinical Pathology 45: 290–296.

Dabai T.J., Zuru A.A., Hassan L.G., Itodo, A.U. and Wara S.H. (2013). Phytochemical Screening of Crude Extracts of *Amblygocarpus* and *Sclerocarya birrea.* International Journal of Traditional and Natural Medicines. 2: 1–8.

Ekhaise F.O., Soroh A. E. and Falodun A. (2010). Antibacterial Properties and Preliminary Phytochemical Analysis of Methanolic Extract of *Ocimum gratissium* (Scent Leaves). Bayero Journal of Pure and Applied Sciences 3: 81–83.

Frassetto L. and Sebastian A. (1996). Age and systemic acid-base equilibrium: Analysis of published data. Journal of Gerontology BIOLOGICAL SCIENCES 51A: B91–B99.

Frassetto L., Morris R.C. Jr. and Sebastian A. (1996). Effect of age on blood acid-base composition in adult humans: Role of age-related renal functional decline. American Journal of Physiology 271: F1114–F1122.

Guyton C. A. and Hall E. J. (2006). Protein Metabolism. In: Textbook of Medical Physiology. 11th Ed. Saunders Publishers. Philadelphia Pennylvania. UK. pp 855.

Jeruto P., Mutai C., Catherine L. and Ouma G. (2011). Phytochemical constituents of some medicinal plants used by the Nandis of South Nandi district, Kenya. Journal of Animal & Plant Sciences. 9: 1201–1210.

Madias N.E., Adrogue H.J., Horowitz G.L., Cohen J.J. and Schwartz W.B. (1979). A redefinition of normal acid-base equilibrium in man: Carbon dioxide as a key determinant of normal plasma bicarbonate concentration. Kidney International 16: 612–618.

Maurer M., Riesen W., Muser J., Hulter H. N. and Krapf R. (2003). Neutralization of Western diet inhibits bone resorption independently of K intake and reduces cortisol secretion in humans. American Journal of Physiology 284: F32–F40.

Muhammad A., Rafiullah M. K., Saqib A., Syed Q. S. and Mamoon U. R. (2013). Antimicrobial properties of extracts and compounds isolated from *Berberis jaeschkeana*. Bangladesh Journal of Pharmacology 8: 107–109.

Ogimoto G., Ozawa S., Maeba T., Owada S. and Ishida M. (1992). ^{31}P-NMR study for the energy metabolism of skeletal muscle in chronic haemodialysis patients. In: Guanidino Compounds in Biology and Medicine, edited by P. P. De Deyn, B. Marescau, V. Stalon, and I. A. Qureshi. London: Libbey. pp. 281–285.

Omage K. and Azeke A. M. (2014). Medicinal Potential of *Acalypha wilkesiana* Leaves. Advances in Research (AIR). 2: 655–665.

Omage K., Azeke A. M., Ihimire I. I. and Idagan A. M. (2013). Phytochemical, Proximate and Elemental Analysis of Acalypha wilkesiana leaves. Scientific Journal of Pure and Applied Sciences. 2: 323–331.

Pierdomenico S.D., DiNicola M., Esposito A.L., Di Mascio R., Ballone E., Lapenna D., Cuccurullo F. (2009). Prognostic Value of Different Indices of Blood Pressure Variability in Hypertensive Patients. American Journal of Hypertension 22: 842–847.

Skeggs L. T. and Hoschstrasser H. C. (1964). Colorimetric determination of chloride. Clinical Chemistry 10: 9I8–920.

Tietz N.W. (1987). Fundamentals of Clinical Chemistry, 3rd Ed. Philadelphia; W.B. Saunders.

Weatherbum M. W. (1967). Phenol-hypochlorite reaction for determination of ammonia. Anal. Chem. 39: 971.