European Journal of Biology and Medical Science Research
Vol.5, No.2, pp.7-19, April 2017
Published by European Centre for Research Training and Development UK (www.eajournals.org)

ELECTROLYTES INDUCED VARIED GLUCOSE LEVELS COMPLICATE EFFICACY OF GLIBENCLAMIDE AND PAW PAW SEEDS EXTRACT IN DIABETES TREATMENT

Jimmy, E. O.[1] and Gilbert, G. E.[2]
[1&2]Department of Physiology Faculty of Basic Medical Sciences University of Uyo, Akwa Ibom State.

ABSTRACT: *The effects of aqueous extract of unripe and ripe paw paw seeds (Carica papaya) were investigated in twenty four (24) alloxan induced diabetes adult rats for 28 days. The results showed that in group 1 rats without diabetes, glucose correlated positively with potassium (r=0.59), but negatively with sodium chloride and calcium; (r – 0.94), (r = -0.84), and (r = -0.65) respectively. The weekly electrolytes levels were not significantly different, (p > 0.05). In group 2, diabetic rats without treatment had raised glucose levels correlated positively with potassium and sodium, (r = 0.32), (r = 0.6) , but negatively with chloride and calcium (r = -0.02), (r = -0.26). Also weekly electrolytes levels were not significantly different, (p>0.05). However, in group 3, diabetic rats treated with glibenclamide, potassium correlated positively with glucose (r = 0.79) but negatively with sodium, chloride and calcium, (r = -.022), (r = -0.1), (r = -0.4). But the weekly electrolyte levels were significantly different, (p<0.05). In group 4 diabetic rats treated with unripe seed extract only glucose levels reduced significantly weekly (p<0.05). The glucose levels correlated positively with potassium, sodium and chloride; (r = 0.84), (r = 0.25), (r = 0.26) but negatively with calcium, (r = -0.77). The weekly electrolytes were not significantly different, (p>0.05) In group 5, diabetic rats treated with unripe seed extract significantly reduced glucose levels weekly, (p<0.05). Potassium, Sodium and chloride correlated positively with glucose (r = 0.3) but negatively with calcium (r = -0.20). But the weekly electrolyte difference was not significant (p>0.05). In group 6 treated with both unripe and ripe seed extract, glucose levels were significantly reduced on day 28 (p<0.05). Potassium, sodium and chloride correlated positively (r = 0.3), but negatively with calcium, (r = -0.8), the weekly electrolyte were significantly different (p<0.05). It is concluded that ripe and unripe paw paw seed extracts are very potent as anti-diabetic therapy but seems to be masked by electrolyte induced raised glucose levels, a complication in the disease.*

KEYWORDS: Electrolyte, Glucose levels, Paw Paw seeds, Diabetes.

INTRODUCTION

Diabetes is a metabolic disorder disease characterized by hyperglycemia and frequent urination. The population attributable to the disease is associated with obesity, age heredity, life style (Mortala, 2004). Globally at least 171 million people suffer from the disease (Wild, 2004). The prevalence rate is partitioned between the urban and the moral dwellers, higher in the former; 65% than the Latter, 35%. The prevalent rate is also found to increase at young age range; 45% (3.3 million) at 45-49 years range and 28%, 26% within the age range of 20, 39 and 79 years (Heine, 2006). In Africa the prevalence is 3.8% with 3.1% in Nigeria (Mclness2012). High rates among the young age is worrisome based on the morality and morbidity consequences of the disease. There are two basic types of diabetes, type 1 and type 2 and perhaps type 3 diabetes as gestational diabetes. The type 1 diabetes is another term for insulin dependent diabetes mellitus (IDDM) which is the child word/juvenile onset diabetes and then

Print ISSN: ISSN 2053-406X(Print), Online ISSN: ISSN 2053-4078(Online)

type 2 another name for non insulin dependent diabetes mellitus (NIDDM). The type 1 is characterized by loss of insulin producing beta cells of the inlet of Langerhans in the pancreas. This type is further complicated with immune status, (Hassan 2012). There is no preventive measure of this type except management in insulin administration. Type 2 diabetes is characterized by insulin resistance with relatively reduced insulin secretion, the reduction is associated with this hormone receptor. Type 1 has 10-15% prevalence of all diabetic cases. The type II is most common, 85% - 95% of the diabetic cases, there is also gestational diabetes, occurring during pregnancy, Guyton 2011,(Ganong, 2013). Diabetes could also result from the genetic defect of beta-cell function, disease of the pancreas or cystic fibrosis, acromegaly and by infections, drugs, chemical effects on the pancreas. The diagnosis of diabetes is based on fasting plasma glucose level of 126mg/dl (7mmol/L) or two hour postprandial plasma glucose (2hr PPG), of 200mg/dL (11.1 mmol/L).

Diabetes is characterized by chronic hyperglycemia its affect protein, carbohydrate and fat metabolism. The clinical symptoms are; polyuria i.e frequent voiding of large volume of urine, polydipsia (increase thirst, polyphagia (increased hunger) and lost of weight. The associated complications are; ketosis, acidosis, ketoacidosis, cardiovascular diseases, neuropathy, retinopathy and foot amputations. (Mclnness 2012). Treatment is the use of insulin therapy but with; set back; insulin resistance, anorexia nervosa, brain atrophy . The use of amylin analogues; is associated with hypoglycemia, visual disorders, tremor, cerebral convulsion, bradycardia, diarrloea thrombocytopenia, hemolytic anaemia, cholestasis hepatitis and jaundice Herbal remedies are effectively use in diabetic treatment, (Sudha, 2009) recently yoyo-bitters have been proven to be very effective in treatment of diabetes (Jimmy 2014).

Electrolytes are key ionic substances in the body that regulate the intracellular and extracellular compartments. Their roles in diabetes become very pertinent to be assayed with respect to sodium and potassium and then related glucose pump,(Guyton 2011). Sodium and potassium are transported actively across the cell membrane via an electronic pump $Na^t(K^t/ATP$. Sodium is most common in the body as sodium chloride, the major cation of the extracellular compartment, Normal range is 136-145mEq/L and normal diet has 5-10g. Sodium maintains electric potential in tissues e.g neuron; brain and nerve functions (Campbell, 1987). High level; hyper natraemia can result in cerebral edema, seizures, brain damage cerebral dehydration and death (Tietz 1990). Hyponatraemia, low sodium level which may result from diahhroea, vomiting, polydipsia, Addison's disease (decreased secretion of aldosterone). System include nausea vomiting malaise headache (Adrogue 2000). Hyponatraemia may lead to diminished reflexes confusion, convulsion above seizures. High potassium level above 6.5mmol/L (hyperkalaemia leads to abnormal heart rhythm; cardiac arrhythmias this can result from lethal infection, and infusion Su(2001), (Leitch, 1994), Treatment involves calcium supplementation which will cause phlebitis and decreases myocardial excitability that protect against arrhythmias. Albutesol and ventolin treatment promote potassium movement into the cells hence lowering the blood levels. Also intra venous insulin injection given with dextrose can shift potassium ions into the cells (with sodium – potassium ATpase pump activity (Mathalahan 2005). Hypokalaemia (low potassium levels) results from low intake, due to heavy fluid loss e.g. diarrhea, excess perspiration, vomiting, urination, loop diuretic (furosemede), amphotericin B, cisplatin, acute rise in plasma bicarbonate (Walmsely, 1984), alkalosis. Raised plasma chloride level; hyperchloremia; chloride concentration exceeding 90-108mEq/L (Sai 2014 (Cambar, 1998). It is caused by dehydration, severe diahrrloea, mineral corticoids, diuretics. Hyperchloremia is associated with renal tubular acidosis, decrease CO_2

Print ISSN: ISSN 2053-406X(Print), Online ISSN: ISSN 2053-4078(Online)

content, over treatment with saline solution, it occurs with acute renal failure and acidosis. However, hypochloremia (low chloride level) is associated with chronic pyelonephsrilis, vomiting and metabolic acidosis, (Tietz 1987). It is also associated with aldosteronism, bromide intoxication, and volume expansion extracellular fluid, (Guyton 2011).

High level of calcium, hypercalcemia leads to depress neuromuscular excitability and cardiac arrhythmias, normal range of calcium is 5mEq/L. Also low level of calcium; hypocalcemia increase nerve and muscle excitability resulting in tetany (Guyton, 2011). Hypocalcemia may be caused by reduction in albumin bound or free fraction (Aurbach, 1992), It is also found in hypoalbuminaemia, chronic renal failure magnesium deficiency hypothyrridism, acute haemorrhagic and edematous pancrititis.

A wide range of herbal preparations are now put forward as antidiabetes therapy. Carica papaya is one of such though with diverse usage but mainly as neutraceutical (Aravind 2013). It is generally called pawpaw, a tropical fruit, often appearing orange red, yellow, green with rich orange pulp. The fruit is delicious and the whole body of the plants including the seeds are explored for their medicinal values. Carica papaya properties also include, vitamins A, B and C, protecolytic enzyme e.g papin, chromopapin with antiviral, antifungal and antibraterial properties. It is used in the treatment of warts, eczema, cutaneous tubercles, glandular tumors, blood pressure, dyspepsia, constipation, amenorrhoea, in deworming, stimulate reproductive organs. In this study the seeds of carica papaya are explored for its antidiabatic potentials as there is no standard therapy for the treatment of this debilitating disease, the likely roles of electrolytes in the disease situation is also considered.

MATERIALS AND METHODS

Extraction of Pawpaw Seeds: The extraction was done according to methods of Trease & Evans, 1996.

The fresh seeds of ripe and unripe pawpaw fruits were air dried for three days and made into powder form. The weight obtained were, 378.2g for ripe powder and 399.1g for the unripe.

350 gram of the unripe powder was dissolved in 200mls of distilled water, stirred and allowed to stand for 24 hours before filtration. It was filtered and the filtrate evaporated for dryness with water bath. The same procedure was done for the unripe seed.

Acute Toxicity Study (LD50)

In the study 30 albino mice were used and the LD50 was determined according to Lorke's method 1983. The test was carried out in two phases. In phase one, the ripe extract the mice were divided into five groups with grouping having three mice. Also each group received the following doses, 5000mg/kg, 4000mg/kg, 3000mg/kg, 2000mg/kg and 1000mg/kg body weight of the extract intrapentonially, (IP) the animals were monitored for signs of toxicity and mortality. In phase two, the unripe, the same number of mice and the grouping were done using fifteen mice altogether. Each group received 5000mg/kg, 4000mg/kg, 3000mg/kg, 2000mg/kg and 1000mg/kg intraperitoneally. The LD50 was determined based on the maximum dose producing O death and minimum dose producing 100% mortality.

$\sqrt{A \times B}$ where A = maximum dose producing 0% mortality
B= minimum dose producing 100% mortality

Print ISSN: ISSN 2053-406X(Print), Online ISSN: ISSN 2053-4078(Online)

Induction of Diabetes with Alloxan

The initial glucose level of each rats were determined using glucometer. Methods of Jimmy et al 2012 were used. The rats were fasted for 24 hrs, a single dose of alloxan (150mg/kg) was administered intraperitoneally as 5% w/v in distilled water freshly prepared. The rats were allowed 72hrs of rest for blood glucose level to stabilize,. Rats with glucose levels between 200-450mg/dL after 72hrs were considered diabetic and were eligible for the study.

Sampling of rats for extract administration

A total of 24 adult albino rats maintained in the Department of Pharmacology animal house with standard pellets and water were randomly divided into six groups of 4 rats per group as follows.

Group 1: As control without diabetes
Group 2: 2nd control with alloxan induced diabetes without treatment
Group 3: Rats induced with alloxan induced diabetes in treated glibendamide
Group 4: Induced diabetes rats treated with ripe pawpaw seed extract.
Group 5: Induced diabetic rats treated with unripe pawpaw seed extract.
Group 6: Induced diabetic rats treated with both unripe and ripe pawpaw seed extract.

1 gram of extract was dissolved in 10ml of distilled water to obtain a stock concentration of 100mg/kg from which concentration per the rats were obtained based on the LD50 per body weight.

The extracts were given to the animals orally using canula by-passing oesphagus into the stomach (Bertram 2004), Robert, 1979. The extracts were administered for 7, 14, 21 and 25 days.

Blood Collection: Blood was collected from the animals through cardiac puncture after anesthesia with chloroform, the blood was spun for 10mins at 2000rpm to obtain the plasma, Dacie and Lewis 2007.

Electrolytes Analysis: The electrolytes analysis was done by the method of Waller, 2000. This is based on the principle that the electrochemical sensor in the equipment converts ion activity into electrical potential of the electrode which relate to NERNST equation which states that the logarithm of the ion activity has a linear relation with electrode potential, (Guyton, 2011). The different electrode is sensitive to different ions e.g. sodium electrode is sensitive to sodium ions and with combining chlorides. The electrolytes are then measured as electrode potentials and processed as concentrations of a particular ion. The results are obtained using this equation which is based on the potentials of the sample and two standard solutions.

$$CX = CA \; EXP \; [EXEA]/S$$

$$S = EB - EA \; / \; Log(CB/CA)$$

Where CX EX: is the concentration and potential of the sample

Print ISSN: ISSN 2053-406X(Print), Online ISSN: ISSN 2053-4078(Online)

CA, EA: Is the concentration and potential of standard A
CB, EB: Is the concentration and potential of standard B
S: Is the slope of electrode

RESULTS

Electrolytes/glucose concentration in non diabetic rats:
The result showed a positive relationship between glucose and potassium (r = 0.59), but negative between glucose and sodium (r = -0.94), glucose and chloride (r = -0.84) table 1, Fig. 1, also negative correlation existed between glucose and calcium, Cl^- = -0.65). This means that as potassium level increases, the glucose level also increase, whereas as Na, Cl, Ca increase the glucose level decreases.

Electrolytes/glucose concentration in diabetic rates without treatment
In this group K (r = 0.32) and Na(r=0.06) were positively related with glucose while chloride and calcium shows a negative relationship Cl^- = -.02) Cl^- = -0.26), therefore as K and Na level increases, the glucose level also increases while as Cl and Na increases the level of glucose decreases, Table 2, fig. 2.

Electrolytes/glucose concentration of diabetic rats treated with glibenclamide
In this group, K^+ showed a positive relationship with glucose (r = 0.79 while Na^+, Cl^- and Ca^+ showed negative relationship with glucose, (r = - .022), (r = - 0.1) (r = - 0.4), Table 3 fig.3.

Electrolytes/glucose concentration of diabetic rats treated ripe pawpaw extract
Glucose had a positive relationship with potassium (r = 0.84), with sodium (r = 0.25) and with chloride (r= 0.26). However, glucose was negatively related with calcium (r=-0.77) the positive relationship between potassium (K), sodium (Na), chloride (Cl) and glucose indicates that as glucose level increases K^+, Na^+ and Cl^- also increases in value but as glucose level increases the Ca^+ level decreases. Table 4, fig. 4.

Electrolytes/glucose concentration of diabetic rats treated with unripe pawpaw seed extract
In this group, a positive concentration existed between K^+, Na^+, Cl^- and glucose levels, (r=0.84), (r=0.25), (r=0.26) but negatively with calcium (r=-0.77), Table 5, fig.5.

Electrolytes/glucose concentration of diabetic rats treated with both ripe and unripe pawpaw seed extract
In this group potassium sodium and chloride correlated positively with glucose, (r=0.4), (r=0.37) (r=0.26) and negatively with r calcium, (r=-0.8). Table 6, fig.6.

Print ISSN: ISSN 2053-406X(Print), Online ISSN: ISSN 2053-4078(Online)

Table 1: Electrolytes/glucose concentration of rats without diabetic

Animals Sacrifices Days	Potassium K(mmol)	Sodium Na(mool)	Chlorine Cl(mmol)	Calcium Ca(mmol)	Glucose Level (Gm/Dl)
7th Day	7.47	142.7	102.1	1.59	96
14th Day	7.01	143.1	103.2	2.75	94
21st Day	5.81	144.5	105.3	2.85	70
28th Day	5.69	144.7	103.1	2.71	78

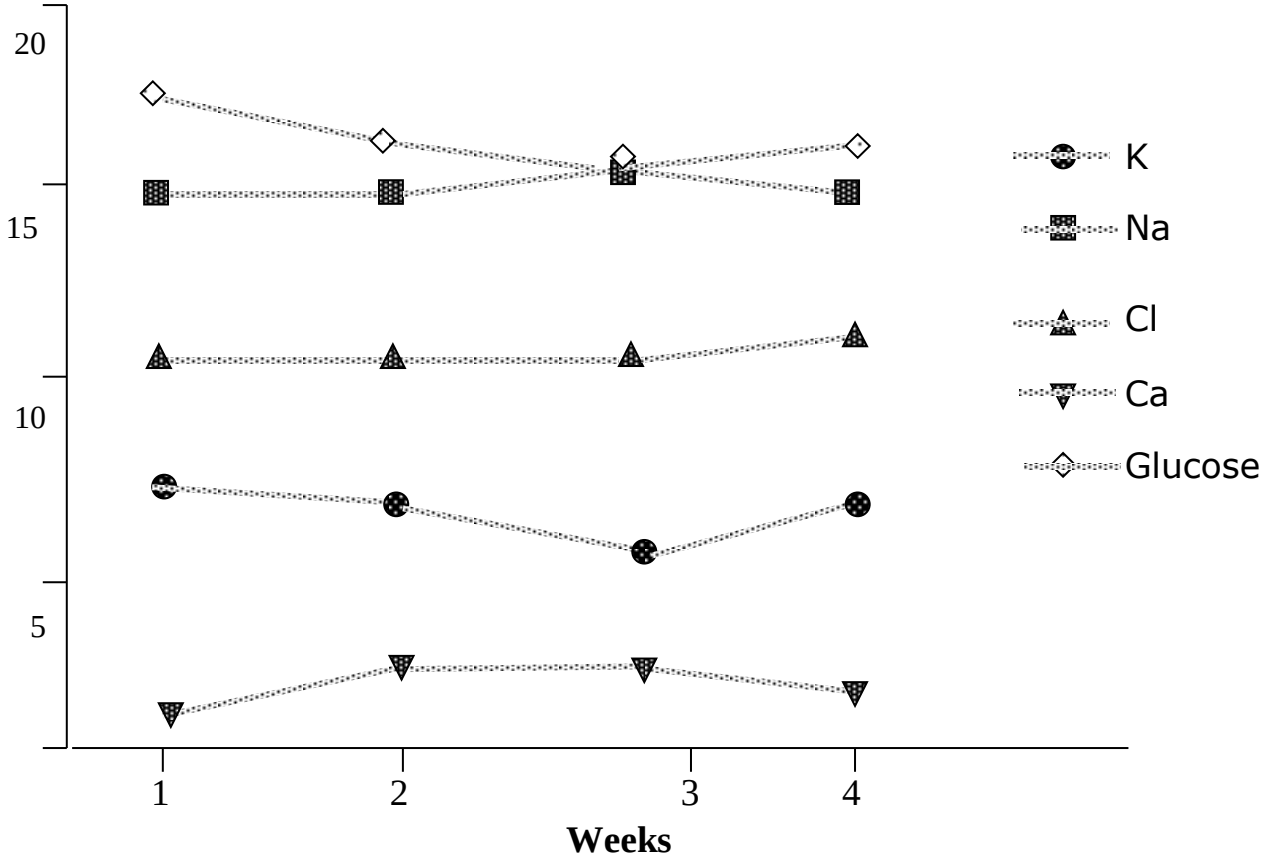


Fig.1

Table 2: Electrolytes/glucose concentration in diabetic rats without treatment

Animals Sacrifices Days	Potassium K(mmol)	Sodium Na(mool)	Chlorine Cl(mmol)	Calcium Ca(mmol)	Glucose Level 9gm/Dl)
7th Day	6.38	114.3	105.1	1.58	162
14th Day	5.88	144.2	105.9	2.68	156
21st Day	4.85	146.3	105.2	2.51	148
28th Day	6.59	140.7	106.7	2.21	147

Print ISSN: ISSN 2053-406X(Print), Online ISSN: ISSN 2053-4078(Online)

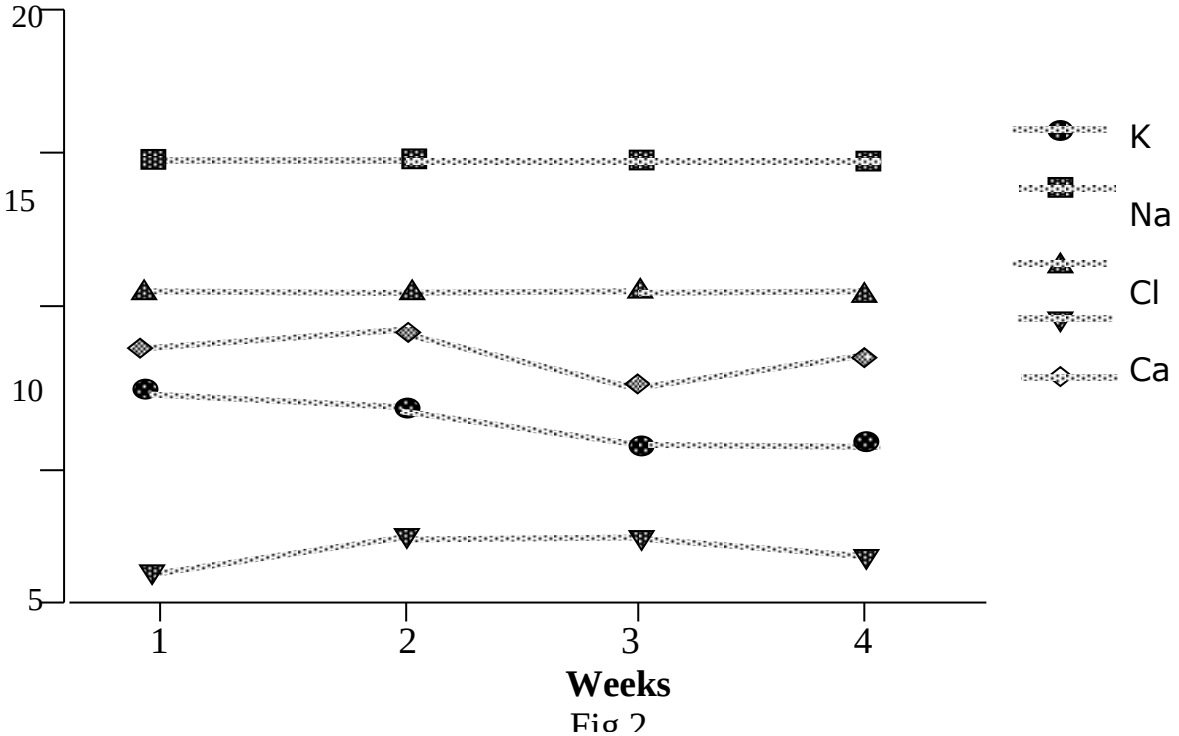


Fig 2

Table 3: Electrolytes/glucose concentration of rats with diabetic treated with glibenclamited

Animals Sacrifices Days	Potassium K(mmol)	Sodium Na(mool)	Chlorine Cl(mmol)	Calcium Ca(mmol)	Glucose Level (Gm/Dl)
7th Day	7.35	142.6	105.5	1.29	342
14th Day	6.68	132.1	92.7	2.45	257
21st Day	5.75	152.5	107.5	2.47	281
28th Day	5.51	145.7	82.5	2.42	173

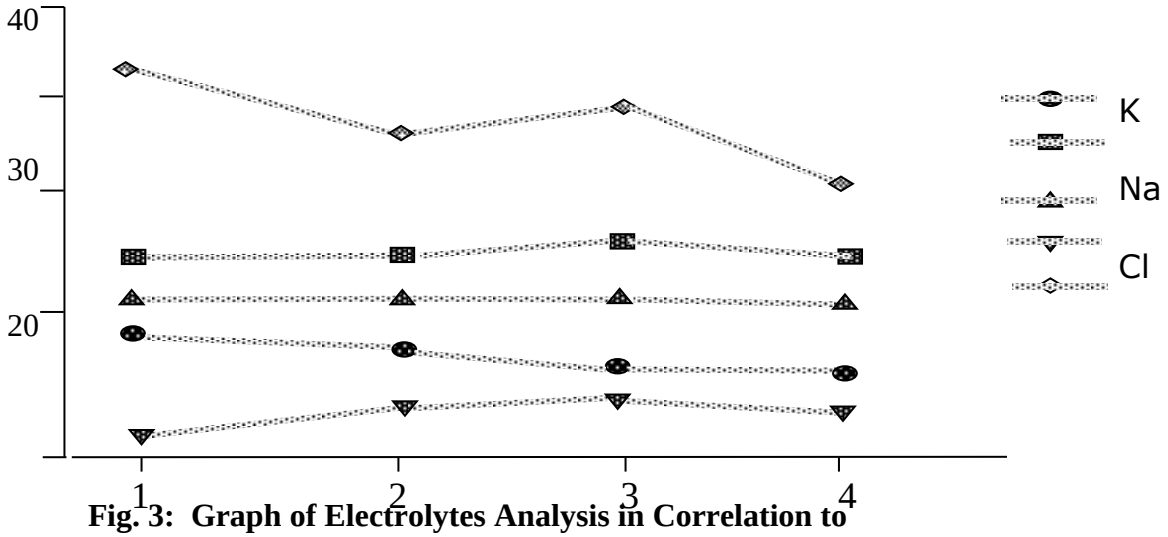


Fig. 3: Graph of Electrolytes Analysis in Correlation to Glucose Level

Print ISSN: ISSN 2053-406X(Print), Online ISSN: ISSN 2053-4078(Online)

Table 4: Electrolytes/glucose concentration of rats
Diabetic rats treated with ripe pawpaw

Animals Sacrifices Days	Potassium K(mmol)	Sodium Na(mool)	Chlorine Cl(mmol)	Calcium Ca(mmol)	Glucose Level (Gm/Dl)
7th Day	5.75	155.0	117.7	2.42	396
14th Day	5.56	121.8	80.9	2.39	320
21st Day	5.59	141.9	102.1	2.67	240
28th Day	5.36	141.6	103.1	2.55	220

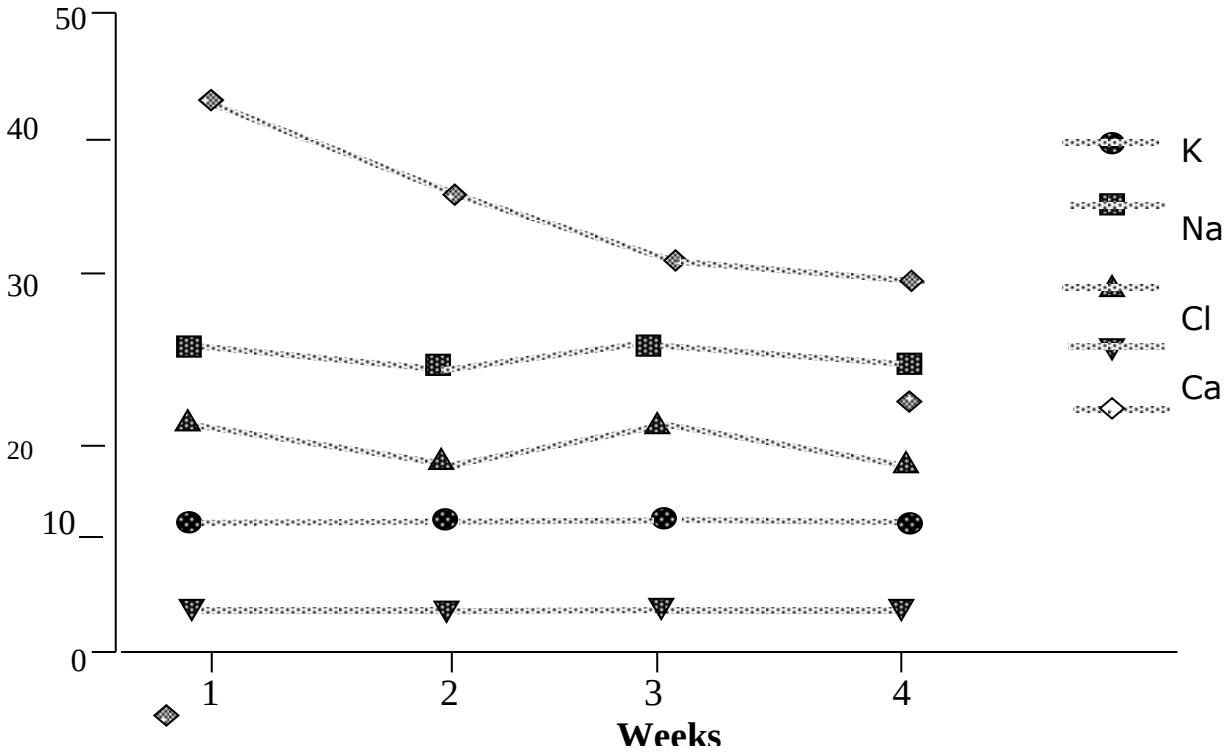
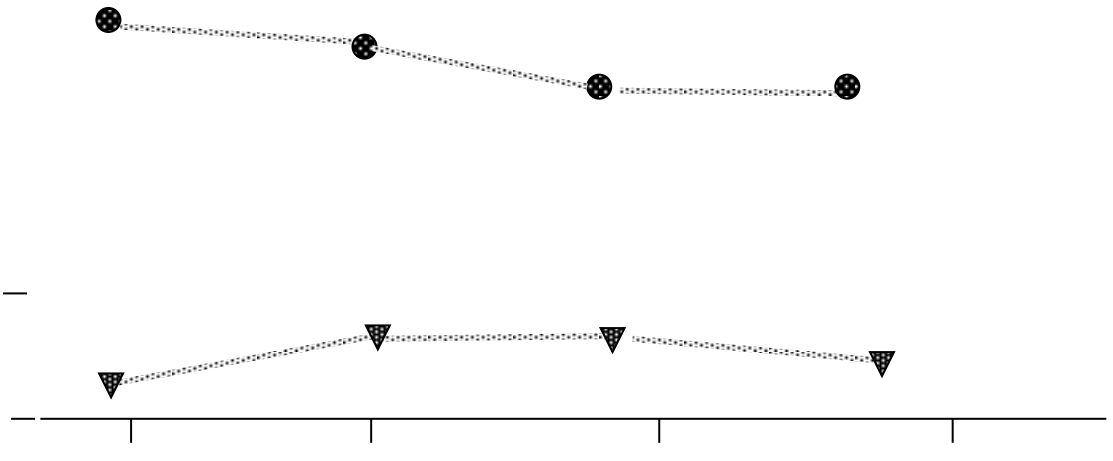


Fig. 4: Graph of Electrolytes Analysis in Correlation to Glucose Level



Print ISSN: ISSN 2053-406X(Print), Online ISSN: ISSN 2053-4078(Online)

Table 5: Electrolytes/glucose concentration of diabetic rats treated with unripe pawpaw seed extract

Animals Sacrifices Days	Potassium K(mmol)	Sodium Na(mool)	Chlorine Cl(mmol)	Calcium Ca(mmol)	Glucose Level (mmol)
7th Day	7.96	139.7	102.2	2.66	300
14th Day	5.58	128.5	88.3	1.75	250
21st Day	6.14	139.3	97.9	2.53	200
28th Day	6.25	134.8	95.6	2.74	183

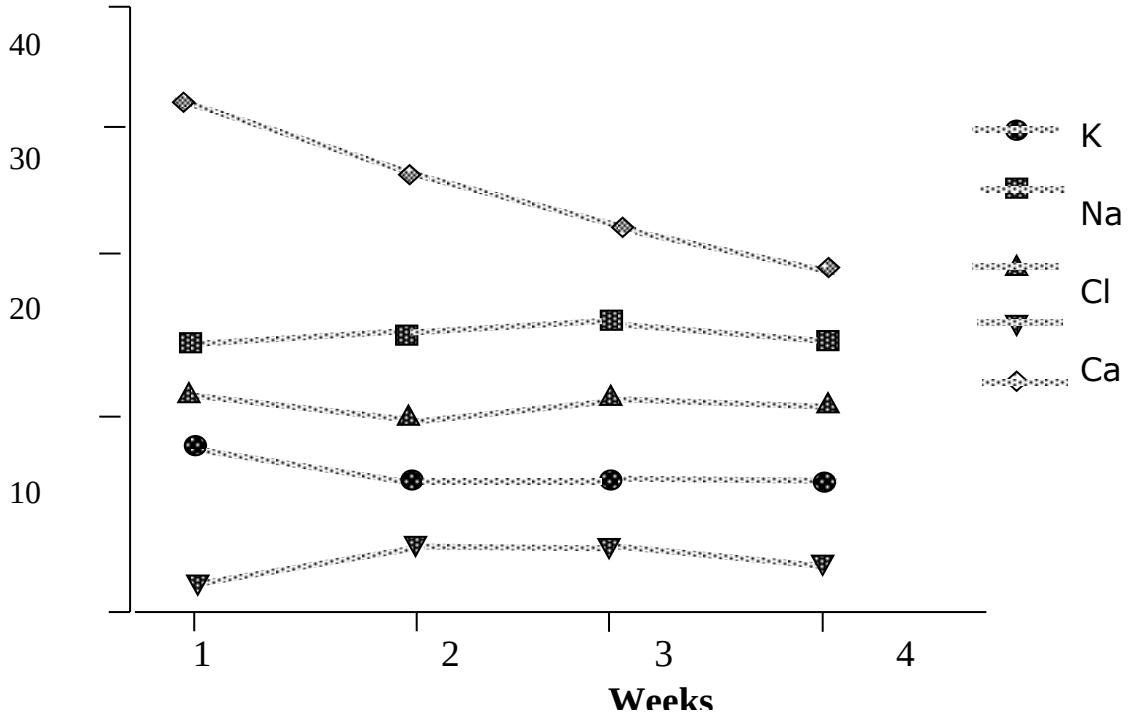


Fig.5: Graph of Electrolytes Analysis in Correlation to Glucose Level

Table 6: Electrolytes/glucose concentration of diabetic rats treated with both ripe and unripe pawpaw seed extract

Animals Sacrifices Days	Potassium K^+(mmol)	Sodium Na^+(mool)	Chloride Cl^- (mmol)	Calcium Ca^{2+}(mmol)	Glucose Level (mmol)
7th Day	6.57	149.0	106.9	1.38	350
14th Day	5.83	124.1	83.1	2.74	220
21st Day	5.70	144.4	105.5	2.55	180
28th Day	6.28	131.5	90.3	2.54	110

Print ISSN: ISSN 2053-406X(Print), Online ISSN: ISSN 2053-4078(Online)

Results presented in table 6 showed a positive relationship between K^+, Na^+, Cl^- and glucose and negative relationship between glucose and Ca^{2+}. Therefore K^+, Na^+, and Cl^- increases as glucose level increases, while the Ca^{2+} decreases as glucose level decreases. These relationships between glucose and electrolytes were not statistically significant.

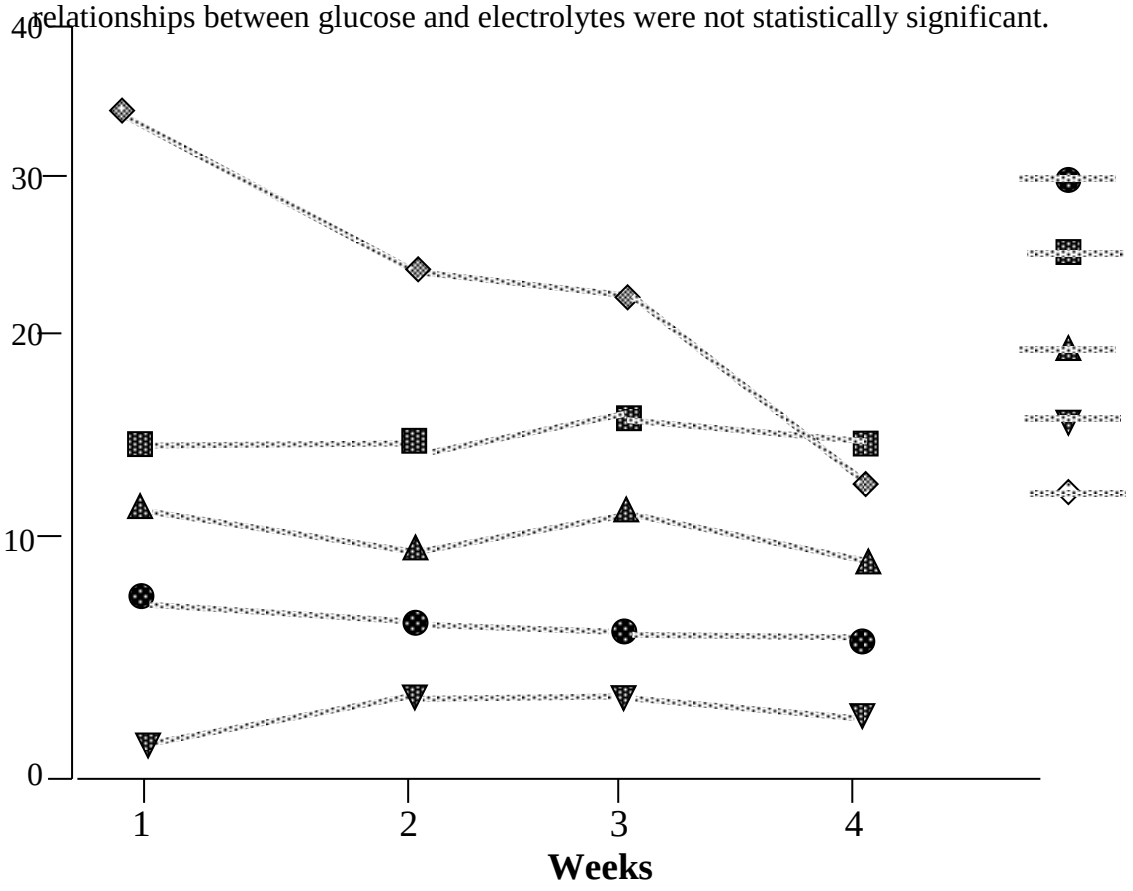


Fig.6: Graph of Diabetes Rats Treated with both Ripe and Unripe Pawpaw Seed Extracts

DISCUSSION

The study has shown the periodic effects of carica papaya seed extract on the plasma electrolytes levels and glucose in diabetes. In the rats with induced diabetes without treatment, potassium level was raised on day 7, dropped slightly on day 14 and 21 but raised higher than all the days on 28 day. The rise in potassium levels correlated with the glucose levels. This indicates the role of glucose as energy source for potassium transport within and outside the cell. But the raised potassium levels hyperkalaemia has also increased the glucose levels as it is correlated positively. This means that high level of potassium adversely affect glucose level and in diabetes it will complicate the diseases situation. Moreover hyperkalaemia leads to cardiac arrhythmias meaning such disease in diabetes, potassium regulate heartbeat and is, (Guyton 2011). Also the sodium levels were slightly raised but correlated negatively with glucose i.e increase in sodium level leads to decrease in glucose level. The decrease in glucose level with increase sodium levels shows the activity of the sodium, potassium pump though not investigated in this study which more of the sodium is enhanced whereas glucose is reduced. Though this mechanism is enhanced by increase on sodium, hypernatrannemia, it is of advantage in diabetic situation as glucose level is reduced with periodic observation. Hypernatremia is associated with thirst, mental confusion, coma and death (wikipedia). This

Print ISSN: ISSN 2053-406X(Print), Online ISSN: ISSN 2053-4078(Online)

also confirms dehydration in diabetes due to frequent urination, (Barry,2010). The chloride levels were raised in induced diabetic rats a condition of hypercholeramia. This is often associated with renal tubular acidosis, decrease CO_2 content and hypokalaemia. It also occurs in acute renal failure, metabolic acidosis, $NaHCO_3$ sodium bicarbonate loss and chronic diarrhea, (Guyton 2011), (Gannong, 2013). Hyperchloremia is a raised level of chloride concentration above 107mEg/L. This condition has been reaffirmed in this study (Cambar, 1998). But the increase in chloride, level was negatively correlated with glucose i.e. as chloride increase, the glucose level decrease from week three i.e 21 days to 28 days. This means that chloride intake could reduce the glucose level in diabetes though such increase has adverse effect. Calcium was also increased in diabetic rats without treatment, an indication of a disease situation, hypercalcemia. This occurs in many conditions, when the influx of calcium into the extracellular fluid is greater than what is extruded. It can also occur when the capacity of the kidneys to excrete filtered calcium is exceeded and also due to increased intestinal absorption of calcium in vitamin D intoxication, and in primary hyperthyroidism (Aurbach, 1992), (Bifexikian, 1990). Hypercalcemia is said to induce wild nephrogenic diabetes insipidus, to present thirst, polydipsia and polyuria, (Adams, 1989) and chronic hypercalcemia may result in depressed muscular excitability and cardiac arrythmias.

The majority of the electrolytes in the group treated with glibenclamide reduced except potassium in the first two weeks of administration. This reduction tallied with reduced glucose level as the days of administration increased i.e to 28 days. This means that glibenclamide also has anti electrolyte properties and for the correlation with glucose confirm that electrolyte enhances the glucose concentration in diabetic disease. But the use of glibenclamide proved less active as compared with ripe and unripe seed extract in the treatment of diabetic rats. The ripe seed extract

CONCLUSION

was more potent than glibenclamide in drastically reducing the glucose level as the period of treatment advanced. However, the unripe seed extract was more potent than the ripe and the glibenclamide. Also the ripe extract reduced the electrolytes levels with corresponding decrease in glucose level as the period of treatment increases. In all, both ripe and unripe pawpaw extract drastically reduced both electrolytes levels and the glucose level in the diabetic rats than the glibenclamide treatment. Generally, it is observed in this study that electrolytes levels complicate the diabetes disease situation and should be monitored in the management of diabetes.

It is observed in the study that raised electrolytes enhanced increase in glucose level. This means that one may have diabetes with increase electrolytes level not because of increase sugar intake or pancreatic abnormally with insulin reduction.

RECOMMENDATION

Diabetic patients should watch their electrolytes diet contents. Also those without the disease should do same. The unripe and unripe pawpaw seed extract are recommended as therapy for diabetes but the glucose and electrolytes levels need be monitored.

Print ISSN: ISSN 2053-406X(Print), Online ISSN: ISSN 2053-4078(Online)

REFERENCES

Adams, J. (1989) Vitamin D Metabolic – Mediated hypercalcemia. Endocrinol. Metab 18:765-778.

Androgue, H J,Madias, N E(2000)Hyponatremia. 342(21)1581-1589

Aravind, G., Bowik, D., Draivel, S. & Harish, G. (2013). Traditional and medicinal uses of *Carica papaya*. Journal of medicinal plant studies, Vol. 1: 7 - 15

Aurbach, G., Marx, S., Speigel., A. (1992). Parathyroid Hormone and the calciferols In: Williams Textbook of Endocrinology, 8th ed. Saunders, Philadelphia pp. 1297-1476.

Bertram, G. (2004) In basic and Clinical pharmacology, 9th edition, New York, New Delhi.

Bilezikan, J. (1990). Calcium and bone metabolism. Principles and Practice of Endocrinology and Metabolism. K. L. Becker ed. Philadelphia, J. B. Lippincott, pp. 368-369.

Cambar, Z., Maraxi, S., Blex, M. and Powel, C. (1998). Diabetes and its relationship with hyperchloremia AMJ Physiol. 231:945-945.

Campbell, N. (1987). Biology. Cunnings Pub. pp.795

Dacie & Lewis (2007) in Practical Haematology practical

Guyton, A. and Hall, J. (2011): In Textbook of Medical Physiology, 12th ed. Elsevier Pub. Philadelphia.

Ganong, W. F. (2013). Review of Medical Physiology, 23rd ed. McGraw Hill.

Heine, J., Diamant, M., Mbanya, J. & Nathan, M(2006) Management of hyperglycemia in type 2 diabetes: the end of recurrent failure. British Medical Journal,333,7580: 1200 - 4

Hassan, G A,Sliem H A, Ellethy, A T., and Salama M S. (2012) Role of immune system modulation in prevention of type 1 diabetes mellitus. Indian journal of endocrinology and metabolism.16 (16) 904-9

Jimmy, E. O., & Okon, M. A. (2012). Periodic Validation of High antidiabetic potentials of unripe plantain in comparison with glibenclamide and fansidar American Journal of Pharmacology and Toxicology 7(1): 15-18.

Jimmy, E. O., and Udofia, A. J. (2014) Yoyo bitters; A potent alternative Herbal drug in the treatment of diabetes. *International of innovative Medicine Health Science*. 23:1-5.

Leitch, S. & Patterson, D. (1994). Interactive effect of potassium, acidosis and catecholamines on isolated rabbit heart, implications for exercise J. Applied Physiol. 77:1164-1171.

Lorke, D (1983) A new approach to practical toxicology testing.*Arch Toxicol*.54.:25-. 287

Mathalahan, T., Macleman, K A., Sandle, L . N,. Verbeke, C., Sandle , G. I.,(2005)

Mortala, A. A., Omar, M. A. K., & Piere, F. J. (2004). Insight into Pancreatitis. *Diabetes* International 10:44-47.

Mclnness, A. D. (2012) Diabetes foot disease in United Kingdom: about time to put feet, *Journal of foot ankles Res*. 5: 26.

Robert, A. (1979) Cytoprotection by prostaglandin gastroenterology 77(**4**)761-767.

Tietz, N. W. (1990): In Clinical Guide to laboratory Test 2nd ed. W. B. Saunders.

Trease and Evans (1996): In Trease and Evans Textbook of Pharmacognosy. Saunders.

Sai C. J. Y(2014) Hyperchloremia Acidosis. Medscape: htt:// emedicine.medscape.com

Sudha A, Murphy V.S and Chandra T S.(2009) Standardization of meta- based herbal medicines. American Journal of infectious Diseases.5: 193-195

Su, M., Stork, C., Ravuri S., Lavoie T,. Anguish, D., Nelson l., Hoffman , R., (2001) Journal of toxicol; clinical Toxicology, 39(6) 641-648

Walmsely, R. & White, G. (1984) Occult causes of hypokalemia. *Clin. Chem*.30 (8): 1406-8

Print ISSN: ISSN 2053-406X(Print), Online ISSN: ISSN 2053-4078(Online)

Wild, S,Roglic, G, Green, A, Sicree , R, & King, H., (2004) Global prevalence of diabetes. *Diacar*e, vol.25, 5; 1047- 1053
Wikipedia: https://en.wikipedia.org

Print ISSN: ISSN 2053-406X(Print), Online ISSN: ISSN 2053-4078(Online)