



Journal of Research in Diabetes & Metabolism

Review Article

Effects of Aqueous Extract of *Ipomoea batatas* Leaf on Blood Glucose, Kidney Functions and Hematological Parameters of Streptozotocin-Induced Diabetic Rats -

Rafiu AA^{1*} and Luka CD²

¹Department of Quality, Nasco Foods Limited, Jos, Nigeria

²Department of Biochemistry, Faculty of Medical Sciences, University of Jos, Jos, Nigeria

***Address for Correspondence:** Rafiu AA, Department of Quality, Nasco Food Limited, Jos, Nigeria, E-mail: akeemrafiu@gmail.com; Carrll42@yahoo.com

Submitted: 20 April 2018; **Approved:** 26 April 2018; **Published:** 28 April 2018

Cite this article: Rafiu AA, Luka CD. Effects of Aqueous Extract of *Ipomoea batatas* Leaf on Blood Glucose, Kidney Functions And Hematological Parameters Of Streptozotocin-Induced Diabetic Rats. J Res Diabetes Metab. 2018;4(1): 004-009.

Copyright: © 2018 Rafiu AA, et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



ABSTRACT

Diabetes mellitus as reported by is a complex disorder that affects metabolism in humans and other subjects, affecting several organs in the system. Management of diabetes is challenging and its treatments are often associated with side effects as scientist is increasingly demanding for natural products with antidiabetic activity and fewer side effects [1,2]. In this study, the effect of aqueous *Ipomoea batatas* leaf extract was carried out in streptozotocin- induced diabetic rats (55 mg/ kg) body wt. for 28days. The continuous administration of extract from 400 mg/ kg body wt. for 28days significantly ($P < 0.05$) reversed the effects on blood glucose which was initially increased. There was a significant decrease ($P < 0.05$) in urea, creatinine, and uric which show kidney impairment following induction of the diabetogen. There was significant increase ($P < 0.05$) in the PCV, Hb, RBC, Neutrophils, Basophils, and Monocytes. While Wbc, platelets, lymphocytes, and Eosinophils were significantly ($P < 0.05$) decreased. The study therefore reveals that aqueous *Ipomoea batatas* leaf extract can be used in hyperglycemia management as well as in improvement in kidney and blood hematological functions.

Keywords: Hyperglycemia; Insulin; Pancreas; High blood sugar; Diabetogen

INTRODUCTION

Ipomoea batatas (sweet potato) leaf is widely used by local communities in Plateau state of Nigeria as a medicinal plant. Sweet potato belongs to the **Domain:** Eukarya **Kingdom:** Plantae; **Phylum:** Magnoliophyta; **Class:** Eudicotyledons; **Order:** Solanales; **Family:** Convolvulaceae; **Genus:** *Ipomoea*; and **Species:** *Ipomoea batatas* [3]. Sweet potato leaves are cooked as a vegetable in many parts of the world. They are rich in vitamin B, carotene, iron, calcium, zinc and protein, and the crop is more tolerant of diseases, pests and high moisture than many other leafy vegetables grown in the tropics and because sweet potato tops can be harvested several times a year, their annual yield is much higher than many other green vegetables [4]. Sweet potato roots and tops possess a variety of chemical compounds relevant to human health. About 80 to 90 percent of sweet potato dry matter is made up of carbohydrates, consisting mainly of starch and sugars with lesser amounts of pectin, hemicelluloses and cellulose [5]. On an average, starch constitutes 60 to 70 percent of the dry matter, but the proportion of starch to other carbohydrates varies greatly. Sweet potato also contains protein (0.46% to 2.93%), dietary fiber (0.49% to 4.71%), lipid (0.06% to 0.48%) and ash (0.31% to 1.06%). It contains essential mineral nutrients such as Ca, P, Mg, Na, K, S, Fe, Cu, Zn, Mn, Al and B. Sweet potato is also an important source of vitamin A, thiamin, riboflavin, niacin, ascorbic acid and many other functional compounds [4,6]. Sweet potato leaves to represent at least 15 anthocyanins and 6 polyphenolic compounds. These biologically active compounds possess multifaceted action, including autoxidation, anti-mutagenicity, anti-inflammation and anti-carcinogenesis [7]. Sweet potato leaves to contain more total polyphenols than any other commercial vegetables, including sweet potato roots and potato tubers [8]. As reported that streptozotocin is a glucose and amine urea compound. Also, the mechanism of streptozotocin action of streptozotocin (STZ, 2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose) is synthesized by Streptomycetes chromogens which is used to induce both insulin-dependent and non-insulin-dependent diabetes mellitus (IDDM and NIDDM, respectively). Recent experiments have proved that the main reason for the STZ-induced B cell death is alkylation of DNA which occurs in the streptozotocin – induced diabetic rats [9,10].

MATERIAL AND METHODS

Plant material

Ipomoea batatas leave was bought from building materials vegetable market, Jos-south Local Government area of Plateau state, Nigeria. The plant root and leaf was identified and verified with a

voucher number (FHJ 232) at the, Herbarium Department, Federal College of Forestry Jos, Plateau state, Nigeria.

Experimental animals

Adult male Wister strains albino rats weighing from 150-200g were used to carry out the study. A minimum of twenty (20) adult albino rats was divided into 4 groups of 5 rats each. The rats were identified as head, back, tail, right hand, and left hand throughout diabetogen induction and plant aqueous treatment. After randomization into various groups and before the start of the experiment, the rats were acclimatized to the animal house condition [11-13]. The rats were maintained on a standard rat feed consisting (70% Carbohydrate, 14.50% protein, 7.0% Fat, 7.20% Fiber and 1.20% mineral) of 28 days and given water *ad libitum*.

Experimental Design

The animal groupings are as follows;

GROUP A- Normal control.

GROUP B- Diabetic control.

GROUP C- Diabetic +Plant extracts (400mg / kg) body weight.

GROUP D Diabetic + Standard drug (Metformin; Rat dose = human dose (500mg)

Rat body weight x 7

Each group consists of four animals, n = 4.

All extracts were at a concentration of 400 mg/kg bwts. For 28 days.

Preparation of extracts

The plant leaf was collected and removed from the stem and air dried at room temperature under shade. The dried plant leaf was pounded on powdery form using pestle and mortar. It was then sieved into a fine powder using mesh size of 180 microns. The powder was stored in an air - tight container until required for use. The preparation of the plant extract was carried out using hot water. 100g of the fine powder was boiled in one (1) Liter of distilled water for 15 minutes (to ensure maximum extractions of phytochemicals) using hot plate. The mixture was allowed to stand for 30 minutes before filtering using atman filter paper No 1 to remove all extractable matters. The filtrate was dried in the autoclave at a temperature of 50 -60°C for two weeks. The solid extract were kept in the refrigerator in an air tight container to be reconstituted in distilled water before use for treatment of diabetic rats.

Administration of plant extracts

The *Ipomoea batatas* aqueous leaf extracts was administered through oral route at a dose of 400mg / kg body weight daily for 28 days. The lethal dose of the plant through oral route is estimated at 12g / kg.

Sample collection

The rats were anesthetized with ethyl ether at 29th day, the neck area was quickly cleared of fur and skin to expose the jugular veins. Blood samples were collected from the animals in batches. Blood samples were separately collected into a clean, dry tube and allowed to clot for 45 minutes and spun at 3000 rpms for 5 minutes for the serum was used for biochemical assay (Blood glucose and kidney function tests). Another Blood sample was separately collected into an anti-coagulant (EDTA) bottle and were used for Hematological assay.

Biochemical parameters

Biochemical parameters assayed are blood glucose determined by method of Serum uric acid was determined by the method of serum creatinine was carried out by the method of serum urea hydrolyzed to ammonia in the presence of ureas in the Berthelot's reaction [14-16].

Hematological parameters

Hematological parameters were determined using Mind ray Hematology Analyzer (Mind ray BC-2800, Guangzhou Shihai Medical Equipment Co. Ltd, China).

Statistical analysis

Data were presented as Mean \pm Standard Deviation (SD) following one-way analysis of variance (ANOVA) using SPSS 20.0 computer software package (SPSS). Differences between were considered significant.

RESULTS

At the end of the period of administration, there was a significant decreased ($p < 0.05$) in the serum glucose level as compared to the normal control rats which was significantly increased after induction of the diabetogen (Table I).

Kidney function

The urea, creatinine, and uric acid shows significant decreased ($p < 0.05$) when compared to the normal control rats following administration of the aqueous plant extract (Table II).

Hematological parameters

Aqueous extracts from the plants increased significantly ($p < 0.05$) the PCV, Hb, and RBC as compared to the normal control rats and the standard drug treated rats (Table III). Neutrophils were significantly ($p < 0.05$) increased, while PLT and WBC were significantly ($p < 0.05$) decreased (Table IV). Lymphocytes and Eosinophils were significantly ($p < 0.05$) decreased but BAS and Monocytes were significantly increased as compared to the normal control rats.

Statistical analysis

Data were presented as Mean \pm Standard Deviation (SD) following one-way analysis of variance (ANOVA) using SPSS 20.0 computer software package (SPSS). Differences between $p < 0.05$ were considered significant.

RESULTS

At the end of the period of administration, there was a significant decreased ($p < 0.05$) in the serum glucose level as compared to the normal control rats which was significantly increased after induction of the diabetogen (Table I).

Kidney function

The urea, creatinine, and uric acid shows significant decreased ($p < 0.05$) when compared to the normal control rats following administration of the aqueous plant extract (Table II).

Hematological parameters

Aqueous extracts from the plants increased significantly ($p < 0.05$) the PCV, Hb, and RBC as compared to the normal control rats and the standard drug treated rats (Table III). Neutrophils were significantly ($p < 0.05$) increased, while PLT and WBC were significantly ($p < 0.05$) decreased (Table IV). Lymphocytes and Eosinophils were significantly ($p < 0.05$) decreased but BAS and Monocytes were significantly increased as compared to the normal control rats.

DISCUSSION

The *Convolvulaceae* is an important family in traditional medicine for the treatment of many ailments The *Ipomoea batatas* leaf which is a member of the family as a vegetable has great economic importance. In the present study, *batatas* leaf extracts is found to have significant effect on serum biochemical parameters; serum glucose, kidney

Table 1: Effects of Aqueous *Ipomoea batatas* leaf Extract on Blood Glucose.

Groups	Treatment	Blood Glucose (Mmol/L)
A	Normal Control	4.05 \pm 0.08
B	Diabetic Control	18.77 \pm 0.32 ^a
C	Diabetic + Extract	6.75 \pm 0.06 ^b
D	Diabetic + Metformin	5.12 \pm 0.02 ^{ab}

NOTE: Values are expressed as Means \pm SD, n = 4; Values in Each Column with Different Letter Superscripts are Significantly Different (P<0.05).

Table 2: Effects of Aqueous *Ipomoea batatas* leaf Extract on Kidney functions.

Groups	Treatment	(Mmol/L)	(Mmol/L)	(μ mol/L)
		UREA	CREATININE	URIC ACID
A	Normal Control	4.69 \pm 0.02	80.00 \pm 0.52	198.78 \pm 0.39
B	Diabetic Control	18.30 \pm 0.79 ^a	347.23 \pm 15.23 ^a	573.13 \pm 5.21 ^a
C	Diabetic + Extract	7.16 \pm 0.01 ^{ab}	143.01 \pm 0.82 ^b	405.78 \pm 1.10 ^{ab}
D	Diabetic + Metformin	5.31 \pm 0.16 ^{ab}	122.16 \pm 0.08 ^{ab}	350.35 \pm 0.81 ^{ab}

NOTE: Values are Means \pm SD, n = 4. Values in Each Column with Different Letter Superscripts are Significantly Different (P<0.05).

Table 3: Effects of Aqueous *Ipomoea batatas* leaf Extract on PCV, Hemoglobin, and RBC.

Groups	Treatment	(%)	(g / dl)	(mm ³)
		PCV	HB	RBC
A	Normal Control	44.75 \pm 0.50	16.25 \pm 0.50	9.79 \pm 0.30
B	Diabetic Control	31.50 \pm 1.29 ^a	10.50 \pm 0.58 ^a	4.65 \pm 0.13 ^a
C	Diabetic + Extract	38.50 \pm 0.58 ^{ab}	13.50 \pm 0.58 ^b	7.48 \pm 0.10
D	Diabetic + Metformin	40.75 \pm 0.96 ^b	12.50 \pm 0.18 ^{ab}	8.24 \pm 0.11

NOTE: Values are Means \pm SD, n = 4. Values in Each Column with Different Letter Superscripts are Significantly Different (P<0.05).

Table 4: Effects of Aqueous *Ipomoea batatas* leaf Extract on PLATELET, WBC, and NEU.

Groups	Treatment	(mm ³) PLT	(mm ³) WBC	(%) NEU
A	Normal Control	167000.00±816.50	6550.00 ±129.10	30.25±0.50a
B	Diabetic Control	2572520.00±1707.83 ^a	10075.00±505.80 ^a	18.00±0.82a
C	Diabetic + Extract	207750.00±957.43 ^{ab}	7512.20±62.92 ^{ab}	25.00±0.00
D	Diabetic + Metformin	186750.00± 500.00 ^b	7125.00±64.55 ^b	25.75±0.50 ^b

NOTE: Values are Means ± SD, n = 4. Values in Each Column with Different Letter Superscripts are Significantly Different (P<0.05).

Table 5: Effects of Aqueous *Ipomoea batatas* leaf Extract on LYM, EOSINOPHILES, BAS and MONO.

Groups	Treatment	(%) LYM	(%) EOS	(%) BAS	(%) MONO
A	Normal Control	65.25±0.50	2.03±0.50 ^a	0.00±0.00	3.00±0.00
B	Diabetic Control	78.00±0.82 ^a	0.50±0.58 ^b	0.50±0.58b	2.00±0.00
C	Diabetic + Extract	71.25±0.96	1.63±0.48 ^a	0.50±0.58	2.50±0.58b
D	Diabetic + Metformin	68.50± 0.58	1.00±1.1	0.75±0.50ab	3.50±0.58b

NOTE: Values are Means ± SD, n = 4. Values in Each Column with Different Letter Superscripts are Significantly Different (P<0.05).

function test, and hematological parameters is true only within limits of dose less than acute toxicity of (12g / kg) of the plant extract. A dose of 400mg / kg body weight of the plant extract from oral route of administration was therefore used to achieve results which signify that *Ipomoea batatas* leaf is safe form consumption within the limit dose administered that certain health benefits of sweet potato leaves are penultimate in anti -diabetic activities, which are the hallmark of this research findings [4] suggested. As seen in Table I, oral administration of aqueous extract of *Ipomoea batatas* leaf to the diabetic rats significantly reduced the blood glucose level compared with the control. Previous reports has indicated that plant extracts possess hypoglycemic properties, possible insulin release stimulatory effects and uptake of peripheral glucose, which in turn reversed the streptozotocin induced hyperglycemia. Hyperglycemia is a characteristic of diabetes which leads to long-term tissue damage and complications, such as liver dysfunctions, often associated with serious diseases [17]. According to glucose can be obtained by hydrolysis of carbohydrates such as milk, cane sugar, maltose, cellulose, glycogen etc. It is however, manufactured by hydrolysis of cornstarch by steaming and diluting acid had reiterated that glucose has a lower tendency than other hexoses to react non-specifically with other amine groups [18]. This reaction to glycan impairs or destroys the function of many proteins. Glucose low rate of glucan as obtained from can be attributed to it having a more stable cyclic form compared to other hexoses, which means it spends less time than they do in its reactive open-chain form. A similar study carried out on diabetogen alloxan –induced rats reveals that the possible mechanism by which aqueous extract brings about its hypoglycemic action may be by induction into pancreatic insulin secretion from β cells of islets of Langerhans or due to enhanced transport of blood glucose to peripheral tissue insulin resistance reducing , inhibition of intestinal glucose absorption, it may contain biomolecules that can modify or stimulate insulin receptors , may modify the structure of glucose transport protein (GLUT 4) and it may inhibit insulin antagonist within the body [19,20]. In the kidney function examination, the urea, creatinine, and uric acid of the streptozotocin- induced diabetic rats are significantly high thus affecting kidney functions as seen in (Table II), when the plant extracts were administered however there

was a significant decreased ($p < 0.05$) from the urea, creatinine, and uric acid ameliorating the impaired effect. This observation is in accord with the reports on [21-23]. Reported that raised plasma creatinine and urea levels in diabetic patients may indicate a pre-renal problem such as volume depletion [22]. In his submission suggested that high creatinine levels observed in diabetic patients may be due to impaired function of the nephrons. According to researchers had commented that high urea levels in diabetes mellitus in patients could be attributed to a fall in the filtering capacity of the kidney thus accumulation of waste products within the system. In addition, a report on the comparative study of serum creatinine levels in male and female type 2 diabetic patients showed that serum creatinine concentration is elevated in type 2 diabetic patients as compared with non-diabetic controls [23]. Also showed reports on a progressive decrease of renal function in male and female diabetic patient as from age 40 years and beyond as a result of increased serum creatinine levels. Male diabetic patients were found to present significantly higher serum creatinine than females. Urea according to literatures is the main part of organic compounds in urine. Urea nitrogen is about 80-90 % of all urine nitrogen and 20-35 g of urea is excreted per day in normal conditions. As stated “Uric acid in approximately 0.6-1.0 g is excreted per day in form of different salts (urates), and mainly in form of sodium salt” [22]. Its amount depends upon food sources. About 1-2 g of creatinine is excreted per day, which is depended on weight of muscles. This is constant for each human. Men excrete 18-32 mg of creatinine per 1 kg of body weight per day, women excrete 10-25 mg per 1kg of their body weight per day. Creatinine is a non-reabsorbable substance, so this test is used for evaluation of renal filtration in kidney functions [24]. Plasma creatinine and urea are established markers of Glomerular Filtration Rate (GFR). Though plasma creatinine is a more sensitive index of kidney function compared to plasma urea level. This is because creatinine fulfils most of the requirements for a perfect marker [25]. In addition a research conducted had found that increase urea and serum creatinine in diabetic rats indicate progressive renal damage [26]. They also found that there is strong correlation between fasting blood sugar and serum urea levels in which the concluded in their study that blood urea and creatinine are accepted to be accessed in renal function as diabetes

mellitus is the major cause of renal morbidity and mortality, so a good control over the sugar level can halt the progression of renal damage [27]. Observed that "serum uric acid, is an end product of purine metabolism that has been shown to be associated with an increased risk of hypertension cardiovascular diseases stated that serum uric acid is also associated with chronic kidney diseases [28,29]. Elevated levels of uric acid is a risk factor of peripheral arterial disease insulin resistance, and components of the metabolic syndrome [27,30]. However, the putative association with serum uric acid levels and diabetes mellitus is not clear. Some studies agree with this present study however that there is a positive association with serum uric acid levels and diabetes mellitus whereas other studies reported no association or an associated relationship as observed serum uric acid has been shown to be associated with cardiovascular disease, hypertension, and chronic kidney disease in previous studies [31,32]. However, few studies have examined the association with serum uric acid and diabetes mellitus and their findings are not consistent examined the association with serum uric acid and diabetes mellitus and found out that higher serum uric acid levels is not directly associated with diabetes mellitus if age, sex, race/ethnicity, education, smoking, alcohol intake, body mass index, hypertension, and serum cholesterol are kept under watch for a diabetic patient [33]. According to as compared to quartile 1 of serum uric acid, the odd ratio (95% confidence interval) of diabetes mellitus was 0.48 (0.35 -0.66) ,the results were consistent by gender and hypertension status in which higher serum uric acid levels were associated with diabetes mellitus in a representative sample of US adults". In Table III and Table V this present study thus revealed that streptozotocin-induced diabetic untreated rats showed some abnormalities in the hematological parameters (PCV, Hb, RBC, Neutrophils, Basophils, & Monocytes) when compared to normal control rats. According to some of these abnormalities in the table III-V might be due to destruction of mature red blood cells, leading to the low Hb counts accompanied by the fall in the RBC and PCV. Administration of the extracts elicits a positive change in the hematological parameters suggesting that it may not contribute further diabetic complications to hematological parameters [34]. The blood is an important body fluid, which contains the Red Blood Cells, White Blood Cells and platelets suspended in the serum in homeostatic concentrations. Blood examination is a good way of assessing the health status of animals as it plays a vital role in physiological, nutritional and pathological status of organisms submitted that assessment of hematological parameters can be used to determine the extent of deleterious effect on blood constituents of an animal [34,35]. Also, hematological parameters were used to explain blood relating functions of chemical compounds plant extract [36]. Peripheral blood leukocytes produce polymorphonuclear cells, including monocytes as well as lymphocytes. Some previous studies showed that peripheral White Blood Cell (WBC) count might be associated with type-2 diabetes, Coronary Artery Disease (CAD), stroke, micro and macro vascular complications [37-39]. Increased differential cell counts, including counts of eosinophils, neutrophils, and monocytes, also indicate the future incidence of Coronary Artery Disease (CAD) [40,41]. Showed that the White Blood Cells (WBC) might play a role in the development and progression of diabetic complications. However, there is no investigation into the differential leukocyte count in relation to diabetic nephropathy. According to a large study of well-characterized non-diabetic subjects with risk factors of type 2 Diabetes Mellitus (DM) had evaluated the associations with hematological parameters which may include Hct, hemoglobin

(Hgb), RBC, WBC with β -cell dysfunction and glucose concentrations [42,43]. In their findings demonstrated that decreasing of neutrophil and hemoglobin levels in the early stage of diabetes may help patients improve their health and reduce their morbidity rate. Cholesterols are essential components of cell membranes including white blood cells and are needed for their shapes and specific functions. However, decreased in WBC levels may help erase the doubt about infection or contamination of the feed during experimental rats administration.

CONCLUSION

It's evident from the study that aqueous *Ipomoea batatas* leaf extracts from 400 mg / kg bwts. Be able to prevent significant increase in blood glucose, kidney impairment and boost blood aberration parameters. The plant extract has proven to be a hypoglycemic therapy for diabetes mellitus and its complications.

REFERENCES

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*. 2002; 27: 1047-1053. <https://goo.gl/65L2wK>
2. Ashok KT, Madhusudana RJ. Diabetes mellitus and multiple therapeutic approaches of Phytochemicals: present status and future prospects. *Current Science*. 2002; 83: 30-38. <https://goo.gl/EH5i4M>
3. Rajapakse S, Nirmalgoda SD, Molnar M, Ballard RE, Austin DF, Bohac JR. Phylogenetic relationships of the sweet potato in *Ipomoea* series *batatas* (convolvulaceae) based on nuclear amylase gene sequences. *Mol Phylogenet Evol*. 2004; 30: 623-632. <https://goo.gl/4xG9mZ>
4. Islam S, Yoshimoto Y, Yamakawa O. Distribution and Physiological Function of Caffeoylquinic Acid Derivatives in Sweet Potato Genotypes. *J Food Sci*. 2003; 68: 111-116. <https://goo.gl/NH3WtY>
5. Scott B, Rose grant B. International Potato Center, and others. 2003; 45-56.
6. Islam S. Sweet potato Leaf: Its Potential Effect on Human Health and Nutrition. *J Food Sci*. 2003; 71: R13-R21. <https://goo.gl/DqVedq>
7. Ishiguro K, Toyama J, Islam S, Yoshimoto M, Kumagai T, Kai Y, et al. Suioh, A New Sweet Potato Cultivar for Utilization in Vegetable Greens. *Acta Hortic*. 2004; 637: 339-345. <https://goo.gl/FYzRai>
8. Brentjens R, Saltz L. Islet cell tumors of the pancreas: the medical oncologist's perspective. *Surg Clin North Am*. 2001; 81: 527-542. <https://goo.gl/5W5h6G>
9. *Diabetes Care*. *Diabetes Care*. The Journal of Clinical and applied research and Education. 2015; 38: S1-S2
10. Dehghan A, van Hoek M, Sijbrands EJ, Hofman A, Witteman JC. High serum uric acid as a novel risk factor for type 2 diabetes. *Diabetes Care*. 2008; 31: 361-362. <https://goo.gl/6PsqEF>
11. Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, et al. Duration of hypertension before initiation of effective antimicrobial. *Crit Care Med*. 2006; 34: 1589-1596. <https://goo.gl/v9dLmR>
12. Miura K, Oda M, Makita S, Chinzei Y. Characterization of the *Drosophila* Methoprene- tolerant gene product. *FEBS J*. 2005; 272: 1169-1178. <https://goo.gl/F3cGwW>
13. Nagappa AN, Thakurdesai PA, Venkat Rao N, Singh J. Antidiabetic activity of *Terminalia Catappa* Linn fruits. *J Ethnopharmacology*. 2003; 88: 45-50. <https://goo.gl/nGTwwQ>
14. Barham D, Trinder P. An Improved Color Reagent for the Determination of Blood glucose by the oxidase system. *Analyst*. 1972; 97:142-145. <https://goo.gl/ke3GtV>
15. Fossati P, Principe L, Bert G. *Clin Chem*. 1980; 26: 227-237.
16. Hare RS. Endogenous creatinine in serum and urine. *Proc Soc Exp Biol Med*. 1950; 74: 148-151. <https://goo.gl/s6Mcvh>
17. Okonkwo PO, Okoye Z. Hypoglycaemic Effects of the Aqueous Extract of *Newbouldia Leaves* Root in Rats. *Int J Biol Chem*. 2009; 12: 42-48. <https://goo.gl/WXYP36>

18. Elsner M1, Guldbakke B, Tiedge M, Munday R, Lenzen S. Relative importance of transport and alkylation for pancreatic beta-cell toxicity of streptozotocin. *Diabetologia*. 2000; 43:1528-1533. <https://goo.gl/fNs7AM>
19. American Diabetes Association, American Diabetes Association. *The Journey and Dream*. 2010; P23.
20. Rafiu AA, Luka CD. Anti-diabetic activity of aqueous seed extract of (*Moringa oleifera*) in normal and alloxan -induced diabetic rats. *Journal of Biological Sciences and Bioconservation* . 2015; 7.
21. Adler I , Stevens RJ, Manley SE, Bilous RW, Cull CA, Holman RR, et al. Development and progression of nephropathy in type 2 diabetes: The United Kingdom prospective diabetes study (UKPDS 64). *Kidney int*. 2003; 63: 225-232. <https://goo.gl/QN78MA>
22. Wagle TJ. Nutrition for reducing urea and creatinine in the blood. *Diabetes care*. 2007; 27:2191- 2192.
23. Wagle T J. Gender wise comparison of serum creatinine and blood sugar levels in type 2 diabetic patients. *Bombay Hospital J*. 2010; 52: 64-68. <https://goo.gl/GQwLUX>
24. Samina HH. *Biochemistry of Specialized tissues (Kidney)*. 2016; 20 -65.
25. Perrone RD, Madias NE, Levey AS. Serum creatine as index of renal function: new insights into old concepts. *Clin Chem*. 1992; 38 :1933-1953. <https://goo.gl/1qmXY6>
26. Anjaneyulu M, Chopra K. Quercetin, an anti-oxidant bioflavonoid, attenuates diabetic nephropathy in rats. *Clin Exp Pharmacol Physiol*. 2004; 31:244-248. <https://goo.gl/UQSWWh>
27. Shankar A, Klein R, Klein BE, Nieto FJ. The association between serum uric acid level and long term incidence of hypertension: population- based cohort study. *J Hum Hypertens*. 2006; 20: 937-945. <https://goo.gl/fE6ST9>
28. Fang J, Alderman MH. Serum uric acid and cardiovascular mortality: the NHANES epidemiologic follow-up study, 1971–1992. *JAMA*. 2000; 283: 2404-2410. <https://goo.gl/CQZcpW>
29. Chonchol M, Shlipak MG, Katz R, Sarnak MJ, Newman AB, Siscovick DS, et al. Relationship of uric acid with progression of kidney disease. *Am J Kidney Dis*. 2007; 50: 239-247. <https://goo.gl/JpG59Q>
30. Yoo TW, Sung KC, Shin HS, Kim BJ, Kim BS, Kang JH, et al. Relationship between serum uric acid concentration and insulin resistance and metabolic syndrome. *Circ J*. 2005; 69: 928-933. <https://goo.gl/FvSYgf>
31. Delaney CA, Dunger A, Di Matteo M, Cunningham JM, Green MH, Green IC. Comparison of inhibition of glucose-stimulated insulin secretion in rat islets of Langerhans by streptozotocin and methyl and ethyl nitrosoureas and methanesulphonates. Lack of correlation with nitric oxide-releasing or O6-alkylating ability. *Biochem Pharmacol*. 1995; 50: 2015-2020. <https://goo.gl/XfWtLf>
32. Taniguchi Y, Hayashi T, Tsumura K, Endo G, Fujii S, Okada K. Serum uric acid and the risk for hypertension and type 2 diabetes in Japanese men: the Osaka health survey. *Journal of Hypertension*. 2001;19: 1209-1215. <https://goo.gl/r8MeST>
33. Pavani B, Anoop S. Association between Serum Uric Acid Levels and Diabetes Mellitus. *Int J Endocrinol*. 2011; 2011: 604715. <https://goo.gl/zgBsZU>
34. Muhammed NO, Adeyina AO, Peters M. Nutritional Evaluation of Fungi Treated Cocoa Bean Shell. *Nigerian J Pure Appl Sci*. 200; 5: 1059-1064.
35. Ashafa OT, Yakubu MT, Grierson DS, Afolayan AJ. Toxicological Evaluation of the Aqueous Extract of *Felicia muricata* Thunb. Leaves in Wister Rats. *African Journal of Biotechnology*. 2009; 8: 949-954. <https://goo.gl/ofYmbw>
36. Yakubu MT, Bilbis LS, Lawal M, Akanji MA. Effect of Repeated Administration of Sildenafil Citrate on Selected Enzyme Activities of Liver and Kidney of Male Albino Rats. *Nig J Pure & Appl Sci*. 2007;18:1395–1400.
37. Ohshita K, Yamane K, Hanafusa M, Mori H, Mito K, Okubo M, et al. Elevated white blood cell count in subjects with impaired glucose tolerance. *Diabetes Care*. 2004; 27: 491– 496. <https://goo.gl/9UagsT>
38. Tong Y1, Shan T, Poh YK, Yan T, Wang H, Lam SH, et al. Molecular cloning of zebrafish and medaka vitellogenin genes and comparison of their expression in response to 17beta-estradiol. *Gene*. 2004; 328: 25-36. <https://goo.gl/D8JNRh>
39. Ford ES. Leukocyte count, erythrocyte sedimentation rate, and diabetes incidence in a national sample of US adults. *Am J Epidemiology*. 2002;155: 57– 64. <https://goo.gl/r5SUAL>
40. Madjid M, Awan I, Willerson JT, Casscells SW. Leukocyte count and coronary heart disease: implications for risk assessment. *J Am Coll Cardiol*. 2004; 44: 1945–1956. <https://goo.gl/rx3iLR>
41. Chung FM, Tsai JC, Chang DM, Shin SJ, Lee YJ. Peripheral Total and Differential Leukocyte Count in Diabetic Nephropathy. *Diabetes Care*. 2005; 28:1710–1717. <https://goo.gl/ossRA2>
42. Hanley AJ, Retnakaran R, Qi Y, Gerstein HC, Perkins B, Raboud J, et al. Association of Hematological Parameters with Insulin Resistance and β -Cell Dysfunction in Nondiabetic Subjects. *J Clin Endocrinol Metab*. 2009; 94: 3824–3832. <https://goo.gl/2EAB2x>
43. Jahangir MD, Sunil CM, Most, NET Mokarrama M, Mominul H, Mehithi H, et al. A comparative analysis of biochemical and hematological parameters in diabetic and non-diabetic adults. *Advanced Medical Sciences: An International Journal (AMS)*. 2015; 2. <https://goo.gl/3LhQAK>