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Evaluating the influence of *Citrullus lanatus* seed extracts on electrolytes, urea and creatinine in Streptozotocin induced diabetic albino rats

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Abstract

The focus of current research study was to evaluate the influence of the seed extract of *Citrullus lanatus* on urea, creatinine, potassium, sodium, chloride and bicarbonate in streptozotocin induced diabetic rats. Eighteen male wistar albino rats were divided into six groups of three rats each, diabetes were induced in all the rats except group 1 by intra-peritoneal injection of 45 mg/kg b.wt. of streptozotocin. Group 1 rats served as control and received standard feed and water daily; Group 2 rats received oral Glibenclamide (0.5 mg/kg bw); Group 3,4, and 5 received 200 mg/kg bw, 400 mg/kg bw and 600 mg/kg b.wt. of the ethanolic extract of *Citrullus lanatus* seed respectively; and group 6 served as diabetic group. Blood samples were collected and analysed for urea, creatinine, potassium, sodium, chloride and bicarbonate using urease-Berthelot colorimetric method, Jaffe's colorimetric method and Ion selective electrode (ISE) method respectively. There was a significant decrease in ($p<0.05$) *glucose co*ncentration (mmol/L) of 3.90 in control compared to 8.07, 8.73, 14.67, 11.43 and 9.80 in albino rats treated with glibenclamide, STZ+CLS 200 mg, STZ+CLS 400 mg, STZ+CLS 600 mg and diabetic control respectively. In potassium concentration (meq/l) 6.51 in control compared with 4.97, 7.59, 7.28, 8.45 and 6.87 in albino rats treated with glibenclamide, STZ+CLS200 mg, STZ+CLS 400 mg, STZ+CLS 600 mg and diabetic control respectively. While in sodium concentration (meq/l) of 1.42 in control compared to 1.33, 1.30, 1.36, 1.42 and 1.36 in albino rats treated with glibenclamide, STZ+CLS 200 mg, STZ+CLS 400 mg, STZ+CLS 600 mg and diabetic control respectively. However, in chloride concentration (meq/l) of 1.06 in control compared to 99.10, 97.87, 1.04, 88.00, 1.04, 88.00 and 1.04 in albino rats treated with glibenclamide, STZ+CLS 200 mg, STZ+CLS 400 mg, STZ+CLS 600 mg and diabetic control respectively and also in bicarbonate concentration (meq/l) of 10.07 when compared with 17.30, 16.47, 15.40, 6.40 and 17.30 in albino rats treated with glibenclamide, STZ+CLS 200 mg, STZ+CLS 400 mg, STZ+CLS 600 mg and diabetic control respectively. In conclusion, this medicinal plant could be considered as a potential and alternative approach for the treatment of diabetes.

Keywords: Keywords: *Citrullus lanatus, Diabetes Mellitus, Renal*

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1. INTRODUCTION

Diabetes mellitus (DM) is a heterogeneous primary disorder of carbohydrate metabolism in which glucose is underutilized. It is characterized by impaired glucose homeostasis with perturbed carbohydrates, fats and protein metabolism as a results of defects in insulin secretion, insulin action or both[1]. DM is a unique and vital disorder due to the highest occurrence rate at global scale[2]. In 2012, an estimated 1.5 million deaths were directly caused by diabetes. More than 80% of diabetes deaths occur in low- and middle-income countries [3]. Diabetic macro-vascular complication is cause high rate of myocardial infarction, peripheral vascular diseases and most important stroke[4]. These risks and their effects are important to study and understand. Due to effects in vessels of blood, it can cause many abnormalities including structural and functional. This includes atherosclerosis, and reduced vascular disease[4]. It is worth to note that fifty percent of diabetics patients expires due cardiovascular disease [5].

DM is conjoined with the presence of micro-vascular impediments. These issues cause peripheral neuropathy and also retinopathy[6]. It Is known now that diabetic peripheral neuropathy cause loss of protective limb mechanical sensations[7]. Also, is found that retinopathy caused by diabetes can lead to blindness and can be due to prolonged damage of small blood vessels of retina[8]. DMA can also cause last stage disease of kidney. It is found that 67% people have nephropathy (diabetic patients)[9]. STZ antibiotic is commonly applied to treat diabetes experimentally[10]. It was observed that STZ usage can cause damage of pancreatic cell membrane, and furthermore, it can spread oxidative stress to islet cells [11,12]. Also, it was found that it can cause breakage of DNA strands and can cause mutation in the pancreatic islet cells[13].

Citrulls lanatus (Watermelon) is nutrient dense food that provides a high concentration of vitamins, minerals and antioxidants for a low amount of calories. It is synonymous with summer and picnics, and for good reason. The refreshing quality and sweet taste aid in combating the heat and also provide a guilt-free, low maintenance dessert for kids and adults alike to enjoy. Watermelons belong to the botanical family Cucurbitaceae. Five types of water melon include seeded, seedless, mini (also known as personal), yellow and orange. *Citrullus lanatus* seed contains phytochemical constituents like alkaloids, flavonoids, tannins, amino acids, carbohydrates, cardioglycosides, terpenoids, oils and fats in the methanolic extract of plant material when compared with other solvents Flavonoids, have been reported to possess antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor and antioxidant activities[14]. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite [15].

The aim of this work was to determine the renal function of streptozotocin induced diabetic albino rats treated with extract of *Citrullus lanatus* using sodium, potassium, chloride, urea and creatinine as indicators.

2. MATERIALS AND METHODS

2.1 Test animals

Forty (40) male wistar Albino rats weighing 129-165g obtained from veterinary farm of the Faculty of Veterinary Medicine University of Nigeria Nsuka, Enugu state were used in this study. The animals were housed in healthy condition, well ventilated stainless steel cage, soft wood shelves bedding in the animal house of Department of Pharmacology/Toxicology Faculty of Pharmacy, Madonna University Nigeria. The rats were allowed to acclimatize for a period of one weeks with free access to water and chall (top-feeds Nigeria LTD) *Adlibitum* .They were kept under standby laboratory conditions(temperature 24-28 ^{0}C), relative humidity 60-70 and 12 hours light/dark cycles. After 7 days, the animals were weighed and randomly assigned to eight groups of five rats each.

2.2 Glibenclamide

A standard anti diabetic drug was purchased from a pharmacy in Port Harcourt.

2.3 Extract Preparation

Fresh Watermelon fruits purchased from local fruit market at Elele Rivers State, Nigeria, weighed and dissected into halves, flesh was removed and seeds collected. The seeds were thoroughly washed, dried in room temperature and milled into a fine powder. It was extracted using 2.5ml of 80% ethanol for 48hours in dark at $25^{0c}$with intermittent shaking. The mixture was filtered first using muslin cloth, through Whattman No.1.filter paper. The filtrate was concentrated in a rotary evaporator for 2 days, the concentrated extract was preserved in a desiccator and stored at 4^{0}C for studies.

2.4 Preparation of high fat diet

The high fat diet was prepared using standard animal feed (top feeds growers mash) sucrose and lard in the ratio3:1:1 respectively. After careful homogenization, the diet was feed to the test animals (except control group) for 2 weeks after Diabetes was induced of Streptozocin. Diabetes was confirmed by measuring blood glucose.

2.5 Animal study

Thirty albino rats were randomly allocated into six groups of five rats each (n=5). Group 1: control; Group 2: Diabetic induced rats 1; Group 3: Diabetic rats treated with Glibemclamide (0.5 mg/kg body weight); Group 4: Diabetic rats treated with 200 mg/kg of *citrullus lenatus* seed extract. Group 5: Diabetic rats treated with 400mg/kg of *citrullus lenatus* seed extract. Group 6; Diabetic rats treated with 600 mg/kg of *citrullus lenatus* seed extract. Diabetes was induced in the test animals after 2 weeks of feeding with high fat diet, the rats were weighed and fasting blood glucose sample was obtained from retrobital sinus under anesthesia with chloroform. A single dose intraperitoneal injection of 45mg/kg of streptozocin (STZ) was administered and the rats were observed for 3days for development of hyperglycaemia. Blood glucose was measured using standard spectrophotometric glucose oxidase method before commencement of treatment. Rat with fasting blood sugar of >11.1mmol/l were used in this study. The administration of extract was totally by gavage. The animals were treated with their respective treatment for 28 days. On the 29th day of treatment and following an overnight fast, the animals were sacrificed under diethyl ether anaesthesia and blood specimens were collected directly from heart (cervical dislocation).Blood sample was collected from each rat into a dry plain sample bottle while 0.5ml of blood was dispensed into Fluoride oxalate bottle for glucose analysis. The blood samples were centrifuged at 2000 RPM for 10 minutes; the respective sera were obtained and stored at 4 ^{0}C for biochemical analysis.

2.6 Electrolytes estimation

Method: ISE analyser

ISE Theory: The analyser utilizes Ion Selective Electrode (ISE) technology. ISE is a type of electrochemical sensor. It converts the ion activity to the electric potential of the electrode. The relation conforms to the NERST equation, that the logarithm of the ion activity has a linear relation with the electrode potential. In addition, different electrode are sensitive to different ions, for example sodium electrode is only sensitive to Na ions and potassium electrode is only sensitive to K ions and chloride electrode is only sensitive to Cl ions. If K electrode, Na electrode and Cl electrode are being combined together, the K ions, Na ions, and Cl ions in the sample can be measured at the same time. The key part of the electrode is the sensitive membrane from the one hand; it is in contact with the sample, responds to the change of the concentration of certain ions in the sample. On the other hand, it is in contact with the internal filling solution, and converts the ionic induction to the electronic conduction through a silver thread i.e. internal electrode. In addition, there is a reference electrode; there is also an internal electrode. Its potential remains constant when the concentration of the solution changes, so it provides a reference point to measure the potential differences.

The instrument measures the electrode potentials, and the data is processed by the microprocessor, to obtain the concentration of a given ion. The measure method is called “standard comparison”. It uses two kinds of standard solutions, one for calibration of the basic point, other for the calibration of the slope. The

result is obtained from the potentials of the sample and two standard solutions. Following are the equation;

$C_X = C_A * EXP [(CE_X - E_A)/S]$ (1)

$$S = \frac{E_B - EA}{Log (CB/CA)} \quad (2)$$

Note:

C_X, EX; The concentration and potential of the sample

C_A, EA; The concentration and potential of the sample A

C_B, EB; The concentration and potential of the sample B

S; The slope of electrode

In order to improve the precision, the contents of the standard solutions should be similar with blood samples as much as possible.

2.7 Monometric method for Bicarbonate (HCO_3^-)

Add certain quantity of blood serum and reagent into a sealed reaction chamber, the HCO_3^- ions in the serum will participate into the reaction and release CO_2, as a result, the gas pressure inside the reaction chamber will be increased accordingly. The pressure sensor detects the changes and sends the signals to the microprocessor to determine the amount if HCO_3^- ion of serum, and then the amount could be displayed and printed [16].

The estimation of Urea was done using Urease - Berthelot colorimetric method. Ten (10) microliter of serum sample, standard, control and distilled water was dispensed into test tubes labelled sample, standard control and blank respectively using Pipette followed by addition of one hundred (100) microliter of urea reagent 1 to all the tubes. The tubes were incubated at 37^{O}C for 10 minutes. Also two hundred and fifty (250ul) microliters each of urea solutions 2 and 3 was added to all the tubes. It was mixed and incubated at 37^{O}C for 15 minutes. The absorbance of the sample, control and standard were read at 546nm against the content of the blank tube. The concentration of unknown sample was calculated by dividing the absorbance of sample against absorbance of standard multiplied by concentration of standard[17].

Creatinine estimation was done by modified Jaffe's colorimetric method. For creatinine determination one hundred (100) microliter of sample was pipetted into test tube containing 1000 millilitre of reagent mixture of Picric acid and sodium hydroxide (500 millilitre each) and absorbance read at 546nm after blanking the spectrophotometer at 1 minute, 2minutes and 3minutes. Also the one hundred (100) microliter of standard was dispensed into test tube containing 1000 millilitre of reagent mixture of Picric acid and sodium hydroxide and absorbance read at 546nm at 1 minute, 2minutes and 3minutes. The concentration of unknown sample was calculated dividing the Changes in absorbance of sample against Changes in absorbance of standard multiplied by concentration of standard[18].

2.8 Statistical Analysis

The data generated were analyzed by one way analysis of variance (ANOVA) using statistical package for social science (SPSS) software. Results are presented as mean ± S.E.M. Values were considered significant at $p<0.05$.

3. RESULTS AND DISCUSSIONS

In table 1 below, the result shows that there was a significant difference ($p<0.05$) in glucose concentration (mmol/l) of 3.90 in control compared to 8.07, 8.73, 14.67, 11.43 and 9.80 in wistar albino rats treated with glibenclamide, STZ+CLS 200 mg, STZ+CLS 400 mg, STZ+CLS 600 mg and diabetic control respectively, in potassium concentration(meq/l) 6.51 in control compared with 4.97, 7.59, 7.28, 8.45 and 6.87 in wistar albino rats treated with glibenclamide, STZ+CLS 200 mg, STZ+CLS 400 mg, STZ+CLS 600 mg and diabetic control respectively, in sodium concentration (meq/l) of 1.42 in control compared to 1.33, 1.30, 1.36, 1.42 and 1.36 in wistar albino rats treated with glibenclamide, STZ+CLS 200 mg, STZ+CLS 400 mg, STZ+CLS 600 mg and diabetic control respectively, in chloride concentration (meq/l) of 1.06 in control compared to 99.10, 97.87, 1.04, 88.00, 1.04, 88.00 and 1.04 in wistar albino rats treated with glibenclamide, STZ+CLS 200 mg, STZ+CLS 400 mg, STZ+CLS 600 mg and diabetic control respectively and in bicarbonate concentration(Meq/l) of 10.07 when compared with 17.30, 16.47, 15.40, 6.40 and 17.30 in wistar albino rats treated with glibenclamide, STZ+CLS 200 mg, STZ+CLS 400 mg, STZ+CLS 600 mg and iabetic control respectively. There was a significant difference ($p>0.05$) in creatinine concentration(µmol/dl) of 52.09 in control compared to 27.01, 31.22, 2.95, 31.55 and 26.52 in wistar albino rats treated with glibenclamide, STZ+CLS 200 mg, STZ+CLS 400 mg, STZ+CLS 600 mg and diabetic control respectively, urea concentration (mg/dl) of 7.27 in control compared to 9.04, 7.77, 15.01, 6.93 and 5.94 in wistar albino rats treated with glibenclamide, STZ+CLS 200 mg, STZ+CLS 400 mg, STZ+CLS 600 mg and diabetic control respectively.

Table 1. Effect of *C. lenatus* on renal function of streptozocin induced diabetic rats

Groups		Glucose (mmol/l)	Creatinine (µmol/dl)	Urea (mg/dl)	Potassim (meq/L)	Sodium (meq/L)	Chloride (meq/L)	Bicarbonate (meq/L)
Control		3.90±0.20	52.09±2.72	7.27±0.64	6.51±0.47	1.42±2.58	106±1.36	10.07±3.43
Glibenclamide		8.07±0.61	270.1±85.00	9.04±0.38	4.97±0.26	1.33±1.19	99.10±0.98	17.30±0.30
STZ+CLS200mg		8.73±1.27	312.2±27.6	7.77±0.12	7.59±0.30	1.30±1.15	97.87±0.94	16.47±0.79
STZ+CLS 400mg		14.67±1.76	295.00±26.8	15.01±1.71	7.28±0.15	1.36±0.58	104±0.29	15.40±0.56
STZ+CLS 600mg		11.43±1.49	315.5±36.3	6.93±0.17	8.45±0.24	1.42±1.19	88.00±5.29	6.40±0.31
Diabetic control		9.80±2.67	265.2±25.5	5.94±0.99	6.87±0.46	1.36+0.93	104±1.09	17.30±2.06
F		5.36	0.97	8.23	12.40	11.53	7.53	7.22
P		0.001	0.470	0.000	0.000	0.000	0.000	0.000
Post Hoc								
Control	Glibernclamide	.069	.045	.453	.325	.298	.207	.595
	200 Citrullus	.251	.043	.990	.629	.150	.084	.688
	400 Citrullus	.108	.974	.167	.785	.536	.813	.794
	600 Citrulus	.152	.087	1.000	.196	1.000	.312	.944
	Diabetic	.563	.019	.999	1.000	.493	.972	.681
Glibernclamide	Control	.069	.045	.453	.325	.298	.207	.595
	200 Citrullus	1.000	.806	.314	.023	.595	.801	.967
	400 Citrullus	.248	.956	.289	.024	.574	.266	.299
	600 Citrulus	.576	.896	.104	.005	.057	.552	.000
	Diabetic	.998	1.000	.817	.193	.708	.353	1.000

200 Citrullus	Control	.251	.043	.990	.629	.150	.084	.688
	Glibernclamide	1.000	.806	.314	.023	.595	.801	.967
	400 Citrullus	.337	.960	.214	.981	.110	.095	.952
	600 Citrulus	.868	1.000	.117	.484	.017	.683	.014
	Diabetic	1.000	.915	.978	.894	.128	.106	1.000
400 Citrullus	Control	.108	.974	.167	.785	.536	.813	.794
	Glibernclamide	.248	.956	.289	.024	.574	.266	.299
	200 Citrullus	.337	.960	.214	.981	.110	.095	.952
	600 Citrulus	.861	.960	.175	.124	.144	.385	.004
	Diabetic	.807	.956	.194	.986	1.000	1.000	.979
600 Citrulus	Control	.152	.087	1.000	.196	1.000	.312	.944
	Glibernclamide	.576	.896	.104	.005	.057	.552	.000
	200 Citrullus	.868	1.000	.117	.484	.017	.683	.014
	400 Citrullus	.861	.960	.175	.124	.144	.385	.004
	Diabetic	1.000	.945	1.000	.292	.119	.373	.138
Diabetic	Control	.563	.019	.999	1.000	.493	.972	.681
	Glibernclamide	.998	1.000	.817	.193	.708	.353	1.000
	200 Citrullus	1.000	.915	.978	.894	.128	.106	1.000
	400 Citrullus	.807	.956	.194	.986	1.000	1.000	.979
	600 Citrulus	1.000	.945	1.000	.292	.119	.373	.138

In table 2 the result shows that there was a significant difference ($p<0.05$) in glucose concentration(mmol/l) of 3.90 in control compared to 8.07, 11.61 and 9.80 in wistar albino rats treated with glibenclamide, *Citrullus lanatus* and diabetic control respectively. There was a significant difference ($p>0.05$) in creatinine concentration (µmol/l) of 52.09 in control compared to 27.01, 119.2 and 43 26.2 in wistar albino rats treated with glibenclamide, *Citrullus lanatus* and diabetic control respectively, also in urea concentration (mg/dl) of 7..27 in control compared to 9.04, 9.91 and 5.94 in wistar albino rats treated with glibenclamide, *Citrullus lanatus* and diabetic control respectively, also in potassium concentration (meq/l) of 6.51 in control compared to 4.20, 7.77 and 6.87 in wistar albino rats treated with glibenclamide, *Citrullus lanatus* and diabetic control respectively, also in sodium concentration (meq/l) of 142.1+2.58 in control compared to 133.3, 135.7 and 135.9 in wistar albino rats treated with glibenclamide, *Citrullus lanatus* and diabetic control respectively, also in chloride concentration of 105.6+1.36 in control compared to 99.9, 96.47 and 103.8 in wistar albino rats treated with glibenclamide, *Citrullus lanatus* and diabetic control respectively, also in bicarbonate concentration (meq/l) of 10.07 in control compared to 17.30, 12.76 and 17.30 in wister albino rats treated with glibenclamide, *Citrullus lanatus* and diabetic control respectively.

The present study determined the effects of the ethanolic extract of the *Citrullus lanstus* (watermelon) seed on blood urea, creatinine, potassium, sodium, chloride and bicarbonate concentrations following streptozotocin induced diabetic in male wistar albino rats.

The result of the study showed significant difference in the glucose concentration of albino rats treated with *C lenatus* at different concentrations compared with their respective controls. This is similar to previous study [19.] A marked decrease in the glucose level in blood was observed when diabetic affected rats were treated with extract. It is reported that *Citrullus lanatus* extract contains flavonoids and polyphenols and this extract have possible hypoglycemic and anti-oxidant potential activities[20]. It is also observed that

arginine in diets has exhibited to decrease the glucose level in diabetic rats[21] possible owing to the Nitric oxide (N O) mediated increase in the blood movement, and increase glucose uptake[22].

Table 2. Effect of *Citrullus lanatus* seed extract on renal function of diabetic induced wistar albino rats.

Groups	Glucose (mmol/l)	Creatinine (µmol/l)	Urea (mg/dl)	Potassium (meq/l)	Sodium (meq/l)	Chloride (meq/l)	Bicarbonate (meq/l)
Control	3.90±0.20	52.09±2.7	7.27±0.64	6.51±0.47	142.1±2.58	105.6±1.36	10.07±3.43
Glibenclamide	8.07±0.61	27.01±0.4	9.04±0.38	4.20±0.26	133.3±1.19	99.9±0.98	17.30±0.30
Citrullus lanatus	11.61±1.15	119.2±88	9.91±1.38	7.77±0.21	135.7±1.76	96.47±2.75	12.76±1.62
Diabetic control	9.80±2.67	26.2±2.55	5.94±2.07	6.87±0.46	135.9±0.64	103.8±1.09	17.30±2.06
F	3.84	0.270	1.200	13.85	2.19	2.05	2.04
P	0.002	0.840	0.350	0.000	0.014	0.015	0.015

		Post Hoc						
Control	Glibernclamide	0.069	0.030	0.304	0.213	0.198	0.126	0.450
	Citrullus	0.002	0.962	0.457	0.310	0.413	0.072	0.961
	Diabetic	0.563	0.010	0.976	0.989	0.358	0.867	0.496
Glibernclamide	Control	0.069	0.030	0.304	0.213	0.198	0.126	0.450
	Citrullus	0.211	0.864	0.987	0.002	0.766	0.785	0.115
	Diabetic	0.998	1.000	0.663	0.121	0.526	0.222	1.000
Citrullus	Control	0.002	0.962	0.457	0.310	0.413	0.072	0.961
	Glibernclamide	0.211	0.864	0.987	0.002	0.766	0.785	0.115
	Diabetic	0.999	0.861	0.578	0.517	1.000	0.158	0.499
Diabetic	Control	0.563	0.010	0.976	0.989	0.358	0.867	0.496
	Glibernclamide	0.998	1.000	0.663	0.121	0.526	0.222	1.000
	Citrullus	0.999	0.861	0.578	0.517	1.000	0.158	0.499

4. CONCLUSIONS

This study exhibited significant difference in the electrolytes (chloride, sodium potassium and bicarbonate concentrations) of albino rats treated with *C lenatus* at different concentrations compared with their respective controls. This is suggestive that *C lenatus* caused changes in the electrolyte content of the albino rats. The result showed that *C. lenatus* caused changes in urea concentration at different concentrations of 200 and 600mg but has no difference in the overall administration according to the result of this study. The result showed that *C. lenatus* caused no changes in creatinine concentration at different concentrations and has no difference in the overall administration according to the result of this study.

CONFLICT OF INTEREST

All authors declare no conflict of interest regarding this article.

REFERENCES

1. Barcelo, A and Rajpathak, S . Incidence and prevalence of Diabetes mellitus in the Americas. American Journal of public Health,2001; 10; 300-308
2. World Health Organization (WHO) (2014) Global status report on noncommunicable diseases. Geneva.
3. World Health Organization (WHO) (2014) Global Health Estimates: Deaths by Cause, Age, Sex and Country. Geneva.
4. Long AN, Dagogo JS . Comorbidities of diabetes and hypertension: mechanisms and approach to target organ protection. J Clin Hypertens, 2011; 13: 244-251.
5. Morrish N, Wang SL, Stevens L, Fuller J, Keen H. Mortality and causes of death in the WHO Multinational Study of Vascular Disease in Diabetes. Diabetologia,2001; 44: S14-S21
6. Deshpande A.D, Harris H.M, Schootman M. Epidemiology of diabetes and diabetes-related complications. Phys Ther., 2008; 88: 1254-1264.
7. World Health Organization (WHO) (2015) Diabetes Fact sheet N°312.
8. Pascolini D, and Mariotti SP. Global estimates of visual impairment: 2010. Br J Ophthalmol, 2012; 96: 614-618.
9. Roglic G, Unwin N, Bennett PH, Mathers C, Tuomilehto J, , Nag S, Connolly V, King H. The Burden of Mortality Attributable to Diabetes Realistic estimates for the year 2000. Diabetes Care, 2005; 28: 2130-2135.
10. Rakieten N, Rakieten ML, Nadkarni MV. Studies on the diabetogenic action of streptozotocin (NSC-37917). Cancer Chemoth. Rep.1963 29: 91-98
11. Bhattacharya G. On the protection against alloxan diabetes by hexoses. Science, 1954; 120: 841-843
12. Schein PS, Rakieten N, Cooney DA, Davis R, Vernon ML. Streptozotocin diabetes in monkeys and dogs, and its prevention by nicotinamide. Proc. Soc. Exp. Biol. Med. 1973; 143: 514-518
13. Matkovics B, Kotorman M, Varga IS, Hai DQ, Varga C. Oxidative stress in experimental diabetes induced by streptozotocin. Acta Physiol. Hung.1997; 85: 29-38
14. Johnson, J.T. Iwang, E.U. Hemen, J.z.Odey, M.O. Efiong, E.E. and Eteng, O.E. Evaluation of anti-nutrient contents of watermelon Citrulluslanatus. Annual Biological Research, 2012; **3**(11):5145-5150.
15. Johnson, I.T. Pokorny, J. N. and Gordon, M.. Antioxidants and Antitumour Properties. In: Antioxidants in Food, (Eds.) 2001. Pp100-123.
16. ISE series operator manual, 2012
17. Weatherburn, M.W. Phenol-hypochlorite reaction for determination of ammonia. *Analytical Chem*istry, 1967; 39: 971-974.
18. Henry R.J. Creatinine in: Clinical Chemistry, principles and techniques.2nd edition, Harper and Row, 1974; Pp525.
19. Kolawole TA, Ojeka SO and Dapper DV. Anti-diabetic effects of the methanolic extract of the rind of *Citrullus lanatus* (watermelon) in alloxan induced diabetes in male albino wistar rats. Journal of Medicine and Medical Sciences,2016; 7(2): 023-029
20. Mowla A, Alauddin M, Rahman A, Ahmed R. Antihyperglycemic effect of *Trigonellafoenum-graecum* (fenugreek) seed extract in alloxan-induced diabetic rats and its use in diabetes mellitus: a brief qualitative phytochemical and acute toxicity test on the extract. Afr. J. Trad. Comp. Alter. Med .2009; 6 (3): 255-261.
21. Kohli R, Meininger CJ, Haynes TE, Yan W, Self JT, Wu G. Dietary L-arginine supplementation enhances endothelial nitric oxide synthesis in streptozotocin-induced diabetic rats. J Nutr., 2004;134(3):600–8.
22. Shi W, Meininger CJ, Haynes TE, Hatakeyama K, Wu G. Regulation of tetrahydrobiopterin synthesis and bioavailability in endothelial cells. Cell BiochemBiophys.2004; 41(3):415– 34.