BENEFICIAL EFFECTS OF *Hibiscus sabdariffa*IN DIABETIC WISTAR RATS

ABSTRACT

*Hibiscus sabdariffa*is a yearly shrub that is widely grown in some Asian and African countries including Nigeria where it is commonly prepared as a hot or cold beverage and also used as medicine. It is applied in folklore remedies in the treatment of some ailments. This study was aimed at investigating the effects of the aqueous calyx extract of *Hibiscus sabdariffa*on some biochemical parameters ofdiabetic male Wistarrats. The rats were divided into four (4) groups of five (5) rats each. Group one (1) which served as non diabetic (negative) control received distilled water only. Group 2 served as diabetic control and received distilled water after alloxan-induced diabetes. Group three (3) and group four (4) were diabetic rats that received 250mg/kg bw and 500mg/kg bw of the aqueous calyx extract of *Hibiscus sabdariffa*respectively, for a period of four (4) weeks. The Statistical Program for Social Sciences (SPSS version 21.0) was used for the analysis. Differences between group means was tested using analysis of variance (ANOVA) and compared using the post hoc test. $P<0.05$ was considered statistically significant.The results obtained showed that the higher dose of the extract caused a significant decrease in blood glucose level in 4 weeks of the study. The changes observed in the plasma concentrations of electrolytes indicated that the extract significantly improved diabetes induced decrease inCl^- level while changes in Na^+, K^+ and HCO_3^-were not statistically significant. It also significantly improved lipid profile and reduced oxidative stress in diabetic male Wistar rats. This study revealed that, the calyx extract of *Hibiscus sabdariffa*demonstrated anti-hyperglycemic effects in diabetic Wistar rats.

Keywords: *Hibiscus sabdariffa*, diabetic Wistar rats, aqueous extract, anti-hyperglycemic.

INTRODUCTION

Diabetes mellitus is a chronic condition defined by persistent hyperglycaemia. It is associated with increased fatiguedue to abnormal glucose metabolism.The disease burden in diabetes is high and still rising in many countries, precipitated by the global rise in the incidence of obesity and unhealthy lifestyles. In 2013, estimates showed a global prevalence of 382 million people suffered from diabetes. It was expected to rise to 592 million by 2035. The two main types are type 1 and type 2 diabetes, with type 2 diabetes accounting for the majority (>85%) of disease prevalence(Forouhi and Wareham.,2014).Persistent and prolonged elevation of blood glucose gradually leads to destruction of most cell types in the body (Oguejiofor et al., 2014) leading to gradual deterioration, and breakdown in function of various organs such as the eyes, heart,

nerves, kidneys and blood vessels [Clinical practice guidelines expert committee (2013);Diabetes Care (2010)].

The development of reactive oxygen species (ROS) may result from persistent elevation in blood glucosecausing oxidative stress and worsening damage to the pancreatic β cells and other organs [Robertson etal., (2003); Pazdroand.Burgess(2010)]. The increase in the incidence of diabetes mellitus has made it a major public health problem calling for special attention towards its control (Whiting et al., 2011). The promotion of public awareness on preventive measures towards curtailing the disease is in the front burner. The present therapies of diabetes mellitus including glucose-lowering drugs such as insulin sensitizers (biguanides,thiazolidine-diones, metformin), insulin secretagogues (sulfonyl-ureas,meglitinides), α- glucosidase inhibitors (miglitol, acarbose) reportedly have reduced efficacy with use and occasionally give rise to harmful side effects such as hypoglycemia,liver injury, channel disturbances, cardiopathyand bloating [Ceriello et al., (2002); Wright et al., (2006)], as well as being considered very expensive especially in sub-Saharan Africa where Nigeria is rated to have the highest number of people with diabetes with researchers reporting a prevalence ranging from 2% to 12% [Nyenweetal.,(2003): Puepet and Ohwovoriole (2008):Sabiretal.,(2011): World Health Assembly (2013); Gezawa (2015)].

This makes the search for new anti-diabetic agents that are cost effective and having fewer or no side effects an important area in drug research. Moreover, people are known to have gained tremendous confidence in traditional and herbal medicine since it has been used in the world for years. In furtherance on the search for alternatives to over the counter drugs, the need for this research study cannot be overemphasized.There is an increasing interest in research on medicinal plants with antidiabetic potential. *Hibiscus sabdariffa*is a medicinal plant that grows as a yearly shrub.It is a genus of the Malvaceae family. Originally from Angola, it is now cultivated in some Asian and African countries including Nigeria. The calyx is used in the production of both hot and cold beverages consumed as refreshment.In addition, its consumption is motivated by its considered medicinal properties (Leung and Foster.,1996).It is applied in folklore remedies in the treatment of certain ailments including diabetes but its efficacy has not been sufficiently proven. This study was aimed at investigating the effects of the aqueous calyx extract of *Hibiscus sabdariffa*on some biochemical parameters such as.blood glucose, lipid profile, selected electrolytes and oxidative stress markers of alloxan induced diabetic male Wistar rats.

MATERIALS AND METHODS

Preparation of Plant Material:

Dried mature red calyces of *Hibiscus sabdariffa* were purchased from the popular Rumuokoro market in Port Harcourt, Rivers state, Nigeria and later identified in the herbarium, Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria(voucher number, UPH/P/254) and a specimen was deposited in the herbarium. The dried calyces were milled to fine powder using a grinding machine. The total quantity obtained was weighed and each 100g was soaked in 400ml of distilled water for 48 hours. The solution was filtered and the extract was concentrated using a rotary evaporator at 45^0C. The net yield was stored in a refrigerator at 4^0C until used. Finally, the extract was reconstituted to obtain 250mg/ml and 500mg/ml of solution for animal oral treatments.

Animal models:

Twenty (20) mature adult male Wistar rats, bred in the experimental animal house of Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria were used for the study.

The animals were housed in clean cages and allowed two weeks to acclimatize with conditions of the housing facility with surrounding temperature of 26-28^0C and adequate ventilation. The animals were fed with standard rat chows and water *ad libitium*. The handling of animals conformed to the guiding principles in the care and the use of laboratory animals published by the American Physiological society (2002).

Experimental design:

The male Wistar rats were divided into four (4) groups of five (5) rats each. Group one (1) which served as non-diabetic (negative) control received normal saline only. Group two (2) served as diabetic control and received normal saline after induction. Group three (3) served as diabetic treated group and received normal saline plus 250mg/kg of extract of *Hibiscus sabdariffa*daily after induction. Group 4 served as diabetic treated group and received normal saline and 500mg/kg of extract of *Hibiscus sabdariffa*daily after induction. The extracts were administered as single oral doses per day using animal feeding hypothermic syringes for four (4) weeks. The

animals were sacrificed under chloroform anaesthesia on day 29 after 24 hours of last administered dose.

Induction of diabetes :

Diabetes was induced by injecting a single (150mg/kg body weight) dose of alloxan monohydrate in normal saline into the rats intra-peritoneally in accordance to the method described by Ebonget al. (2008). Blood samples were collected by caudal venepuncture after 72 hours and the glucose level was measured using the glucometer. The rats with blood glucose level above 200 mg/dl were considered diabetic and included in the study.

Blood glucose was monitored weekly throughout the period of experiment and on the last day of experiment.

Collection of blood and analysis:

Blood samples were collected at the end of the experiment into appropriate sample bottles.

For estimation of serum lipid profile [Total cholesterol (TC), Triglyceride, High density lipoprotein-cholesterol (HDL-c)], blood was collected through cardiac puncture into appropriate sample tubes. After collection, the samples were centrifuged for fifteen minutes in a serologic electric centrifuge [Centrifuge 80-2 (Techmel&Techmel USA)]. Then, 1 mL aliquots of serum were removed and used to perform the biochemical assay. In analysing biochemical parameters, commercial Labtest Diagnostic kits with standard techniques based on enzymatic and colorimetric methods, spectrophotometry and the use of a semi auto-analyser (Contec 800 A) manufactured by Randox, USA, in accordance to the manufacturer's recommendations. The concentrations were determined by use of an automatic biochemical analyzer. The determination of Low density lipoprotein-cholesterol (LDL-c) was calculated using the Friedewald formula. .

Electrolytes analysis was done using SFRI 4000 ion selective electrode.

Pancreatic islet tissue sample was used for the estimation of oxidative stress biomarkers in accordance with standard methods [Goldberg and Spooner (1983); Wasowiczetal., (1993); Slaughter and O'Brien (2000);Vives-Bauzaetal., (2007);Condezo-Hoyosetal., (2013); Peskin and Winterbourn,(2017)]

Qualitative phytochemical screening of *Hibiscus sabdariffa*

The aqueous calyx extract of *Hibiscus sabadariffa* was qualitatively screened for the presence of secondary metabolites to relate some and Biochemical properties of extract in diabetic conditions

with the presence or absence of these constituents. Phytochemical constituent measured were as follows;

Tests for alkaloids, flavanoids, carbohydrates, saponins, glycosides, tannins and anthraquinnones were done in accordance with established methods [Cannel, (2000). Trease and Evans, (2002); Adegoke et al., (2010);Harbourne, (1984)]

Ethical clearance

Institutional ethical approval was obtained for this study from the ethical committee of the Research Ethics and Development center at the University Of Port Harcourt, Nigeria with certificate no; UPH/CEREMAD/REC/MM77/066.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) version 20.0 was used for the statistical analysis of data. Results were expressed as Mean ± SEM. The means were determined using the one-way analysis of variance (ANOVA), compared using post hoc LSD and considered statistically significant at $p<0.05$.

RESULT

Result presentation

The result of this study are presented in tables 1-6.

Table 1:Blood glucose level of Wistar rats

Values expressed as mean±SEM. n=5. *,#, Significantly different when compared to negative and diabetic controls respectively at (P<0.05).

Table 2: Total cholesterol and LDL levels of Wistar rats after *Hibiscus sabdariffa* extract administration

Groups	Blood Glucose (At induction) (mg/dl)	P-Value	Blood glucose (4weeks) (mg/dl)	Change in blood glucose (mg/dl)	P-Value
Negative Control	86.40±6.81	-	91.44±2.50	5.04±5.04	-
Diabetic Control	345.60±30.86*	0.00	255.60±31.89	-90.00±20.52	-
250mg/kg	206.64±34.37*	0.01	84.24±9.15	-122.40±28.09	0.31
500mg/kg	324.36±43.22*	0.00	106.20±20.99	-218.16±23.25#	0.00

Values expressed as mean±SEM. n=5. #,Significantly different when compared to diabetic control at (P<0.05)

Groups	TC (mmol/l)	P-value	LDL (mmol/l)	P-value
Negative Control	2.88±0.16	-	1.77±0.12	-
Diabetic Control	3.08±0.04	-	1.86±0.12	-
250mg/kg	2.92±0.25	0.32	1.34±0.09#	0.00
500mg/kg	2.76±0.07#	0.04	1.48±0.10#	0.02

Table 3: Triglycerides and HDL levels of Wistar rats after *Hibiscus sabdariffa* extract administration

Values expressed as mean±SEM. n=5. #,Significantly different when compared to diabetic control at (P<0.05)

Groups	TG (mmol/l)	P-value	HDL (mmol/l)	p-value
Negative Control	1.12±0.07	-	1.62±0.07	-
Diabetic Control	1.26±0.03	-	1.58±0.11	-
250mg/kg	1.26±0.04	0.32	2.10±0.17$^{\#}$	0.01
500mg/kg	1.36±0.12	0.05	2.02±0.11$^{\#}$	0.03

Groups	GSH (µg/ml)	CAT (µ/g)	P-value	SOD (µ/ml)	MDA (µmol/ml)	P-value

Table 4: Blood electrolytes of Wistar rats after *Hibiscus sabdariffa* extract administration

Values expressed as mean±SEM. n=5. *,$^{\#}$Significantly different when compared to negative control and diabetic control at (P<0.05) respectively.

Groups	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	P-value	HCO_3^- (mmol/l)
Negative Control	133.20±3.65	4.52±0.29	68.00±0.71	-	25.60±1.33
Diabetic Control	137.20±2.22	4.42±0.22	62.20±0.86*	0.00	26.00±1.41
250mg/kg	132.00±0.84	4.24±0.22	66.00±1.58$^{\#}$	0.01	25.60±1.72
500mg/kg	135.80±1.42	4.58±0.12	67.00±1.00$^{\#}$	0.00	27.60±0.79

Table 5: Oxidative stress markers of Wistar rats after *Hibiscus sabdariffa* extract administration

Negative Control	1.37±0.21	3.17±0.20	-	0.22±0.02	0.53±0.02	-
Diabetic Control	1.06±0.02	3.16±0.20	-	0.16±0.01	0.61±0.01	-
250mg/kg	1.44±0.28	4.13±0.45[#]	0.04	0.21±0.03	0.38±0.05[#]	0.00
500mg/kg	1.23±0.18	4.79±0.50[#]	0.00	0.26±0.02	0.27±0.05[#]	0.00

Values expressed as mean±SEM. n=5. [#],Significantly different when compared to diabetic control at ($P<0.05$)

Table 6: Qualitative phytochemical analysis of aqueous calyx extract of *Hibiscus sabdariffa* to indicate presence or absence of substance.

Parameter	**Indicator**
ALKALOID TEST	+
CARBOHYDRATE TEST	+
FLAVONOIDS TEST	+
SAPONIN TEST	+
ANTHRAQUINONES TEST	-
TANNINS TEST	+
GLYCOSIDES TEST	+

Present (+) ; Absent (-)

DISCUSSION

Scientific evaluation of medicinal plants employed in folklore management of diabetes mellitus is important in order to confirm or disprove any report of their medicinal benefit in treatment of

diabetes mellitus. In this study, blood glucose level significantly increased following induction with alloxan in all diabetic groups (Groups 2-4) when compared to negative control group. In a report,induction of diabetes occurred on injection of alloxanin rats that received no other form of drug treatment during the study (Ebong.,2008).Alloxanhas the capacity to cause damage and death of the insulin-secreting pancreatic cells in experimental animal models that is responsible for the resultant hyperglycaemia and diabetes (Lenzen, 2008). Post treatment blood glucose level (after 4 weeks) showed a significant reduction ($P< 0.05$) in the group that received the higher dose (500mg/kg) of the extract compared to diabetic control. The hypoglycemic properties of plants may be due to their ability to stimulate possible insulin release and uptake of peripheral glucose (Okonkwo and Okoye, 2009).This may indicate that the extract at the higher dose may have been able to stimulate the regeneration of the beta cells of the pancreas, which in turn, reversed alloxan induced hyperglycemia.

The finding in this study is in agreement with the reported findings in a similar study that indicated that *Hibiscus sabdariffa* calyx extract caused significant ($p < 0.05$) reduction in blood glucose level (Adefolalu et al., 2019).

The result also reveals a significant ($P<0.05$) improvement in lipid profile. The higher dose of the extract caused a decrease in total cholesterol, while both doses significantly reduced LDL-c and significantly increased HDL-cafter administration of the *Hibiscus sabdariffa*extract.

This finding is in agreement with reported observations in a similar research study (Patrick et al., 2014) that stated that *Hibiscus sabdariffa*, a hypoglycaemic agent, reduced total cholesterol and LDL-c levelin extract-treated groups when compared to diabetic and non-diabetic controls. The decrease in LDL-c could be due to the inhibition of the triacylglycerol synthesis or other hypolipidemic effects, through the antioxidant mechanism that prevent oxidation of LDL-c and hepatic liver clearance. Prolonged abnormally elevated blood glucose level in diabetes mellitus may lead to damage in some tissues and organs such as the kidney giving rise to renal insufficiency. In this study, plasma electrolytes such as,sodium, potassium, chloride and bicarbonate ions; were assayed to assess the effects of extracts of *Hibiscus sabdariffa*in any possible diabetes induced renal dysfunction in diabetic rats treated with same extract.The mean plasma sodium, potassium and bicarbonate ion levels were not significantly ($P<0.05$) altered in this study, when the treated diabetic and non treated diabetic (diabetic control) groups were

compared to the non diabetic (negative) control group and when the treated diabetic groups were compared to the non treated diabetic control group at the end of four weeks of study. However, the chloride ion levels were significantly reduced in the diabetic group compared to negative control. But the mean chloride ion level was improved in the abstract treated groups compared to the diabetic control group implying that the abstract of *Hibiscus sabdariffa* protected against diabetes induced reduction in serum chloride ion level.However, the degree and duration of persistent hyperglycemia may determine the onset of organ damage especially, the kidney. The serum electrolytes as well as blood urea and creatinine are bio-markers used to assess and monitor renal function in diabetics with poorly controlled diabetes (Bamanika et al.,2016).The glomerular filteration, tubular reabsorption and tubular secretion are important roles of the kidney which also reflect their functional state.Several mediators in the form of reactive oxygen species are responsible for the cell destructive action of alloxan. Alloxan and dialuric acid, a product of its reduction sets into motion a series of actions leading to the generation of reactive oxygen species and ultimately the rapid destruction of pancreatic β-cells, thereby, precipitating experimental diabetes mellitus (Lenzen, 2008). Reactive oxygen species generated spontaneously in cells during metabolism causes degradation of polyunsaturated lipids leading to the production of malondialdehyde (Pryor and Stanley.,1975) ,which serves as a biomarker to measure the level of oxidative stress in an organism(Moore and Roberts.,1998). The malodialdehyde production reduced significantly in the extract treated groups while catalase enzyme activity improved significantly in the group that received lower dose of the extract. The antioxidant action of *Hibiscus sabdariffa*may be due to its ability to inhibit lipid peroxidation by the removal of free radical intermediates. In this study, the change in these oxidative stress markers suggest that the extract of *Hibiscus sabdariffa*may play important roles in preventing toxic stress in pancreatic β-cells.

The medicinal properties of the plant are derived from its phytochemical constituents. Several bioactive compound such as tannins, saponins and flavonoidsare present in the calyx extract of *Hibiscus sabdariffa.*The plants antioxidant property may be due to the flavonoids content. Flavonoids are reportedly good antioxidants (Shrivastava et al., 2012),which may possess the capacity to control or prevent oxidative stress and associated disorders. Flavonoids and phenolic compounds are diabetes induced free radical scavengers which are also associated with ability to trigger regeneration of damaged pancreatic beta cells in diabetic rats and increase insulin

secretion (Chakravarthy., 1980). In a study, it was reported that saponins also possess blood glucose lowering effects (Li et al., 2002). The major bioactive constituents of *Hibiscus sabdariffa* are important in the context of theirpharmacological effects as antioxidant, hypocholesterolaemic and hypoglycaemic agents.

CONCLUSION

The extracts of *Hibiscus sabdariffa* possess antidiabetic, antioxidative and anticholesterolaemic effects; and also improves renal function test in alloxan induced diabetic Wistar rats.In addition, phytochemical constituents of the extract including flavonoids, saponins, tannins etc.hasbeen shown to be responsible for its range of pharmacological effects.

REFERENCES

1. Adefolalu FS, Salawa JS, Gara TY (2019). Abubakar Hypoglycemic Effect of Methanol Extract of Hibiscus Sabdariffa Seed in Alloxan Induced Diabetic Albino Rats Nigerian *Journal of Basic and Applied Science* 27(2): 151-156
2. Adegoke A, Iberi P, Akinpelu D, Aiyegoro O, Mboto C. (2010). Studies on phytochemical screening and antimicrobial potentials of Phyllanthusamarus against multiple antibiotic resistant bacteria. *International Journal of Applied Research in Natural Products*;3(3):6–12.
3. American Physiological Society.(2002). Guiding principles for research involving animals and human beings. *Am. J physiol.Regul.Integr Comp Physiol.,* 283: 281-283
4. Cannel R. (2000). Methods in Biotechnology. Natural Products Isolation. Human press, New Jersey.
5. Ceriello A, Quagliaro L, Catone B, (2002). Role of hyperglycemia in nitrotyrosine postprandial generation. *Diabetes Care* 25: 1439–1443.
6. Chakravarthy BK, Gupta S, Gambir SS and GodeKD .(1980). Pancreatic beta cell regeneration: A novel antidiabetic mechanism of *Pterocarpusmarsupium*Roxb, *Ind. J. Pharmacol*., 12:123-127.
7. Clinical practice guidelines expert committee (2013). Diagnosis, classification, and diagnosis of diabetes, pre-diabetes and metabolic syndrome. *Can Journal of Diabetes*;37:S8-11.

8. Condezo-Hoyos L, Rubio M, Arribas SM et al.,(2013). “A plasma oxidative stress global index in early stages of chronic venous insufficiency,” *Journal of Vascular Surgery*. 57(1):205–213.

9. Diabetes Care (2010).Diagnosis and classification and diagnosis of diabetes mellitus. *Diabetes Care* ;33:S62-9.

10. Ebong PE, Atangwho IJ, Eyona UE and Egbung GE. (2008).The anti-diabetic efficiency of combined extracts from two continental plants: *Azadirachtaindica*(A.Juss) (Neem) and *Vernoniaamygdalina*(Del.) (African bitter leaf). *Am.J. Biochem. Biotechnol*., 4: 239-244

11. Forouhi NG, Wareham NJ .(2014). Epidemiology of diabetes. Medicine (Abingdon). Dec;42(12):698-702. doi: 10.1016/j.mpmed.2014.09.007. PMID: 25568613; PMCID: PMC4282306.

12. Gezawa ID, Puepet FH, Mubi BM, Uloko AE, Bakki B, Talle MA, et al. (2015). Socio-demographic and anthropometric risk factors for type 2 diabetes in Maiduguri, North-Eastern Nigeria. Sahel Med J;18(5):1–7.

13. Goldberg DMand Spooner RJ. (1983). “Assay of glutathione reductase,” in Methods of Enzymatic Analysis, H. V. Bergmeyer, Ed., pp. 258–265, VerlagChemie, Weinheim, Germany.

14. Harbonre JB. (1984).Phytochemical Methods. A guide to modern techniques of plant analysis. Chapman and Hall Limited, London, ; 40-75.

15. Lenzen S. (2008). The mechanism of alloxan and streptozotocin induced diabetes.*Diabetologica*, 51:216-226.

16. Leung A, Foster S, eds.(1996). Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics. 2nd ed. New York: John Wiley and Sons.

17. Li M, Qu W, Wang Y, Wan H and Tian C. (2002). Hypoglycemic effect of saponin from Tribulusterrestris, Zhong Yao Cai, 25 (6), 2002, 420-2.

18. Moore K and Roberts LJ .(1998). Measurement of lipid peroxidation. *Free Radic. Res.*, 28(6): 659-671.

19. Nyenwe EA, Odia OJ, Ihekwaba AE, Ojule A, Babatunde S. (2003). Type 2 diabetes in adult Nigerians: a study of its prevalence and risk factors in Port Harcourt, Nigeria. *Diabetes Res Clin Pract*;62(3):177–185.

20. Oguejiofor O, Odenigbo C and Onwukwe C (2014). Diabetes in Nigeria: Impact, Challenges, Future Directions.*Endocrinology& Metabolic Syndrome* ,3:2.

21. Okonkwo PO and Okoye ZSC.(2009). Hypoglycaemic Effects of the Aqueous Extract of Newbouldia Leaves Root in Rats, *Int. J. Biol. Chem*.12,2009: 42-48.

22. Patrick EA, Nwaigwe CU, Okwuagwu FO, Udem SC, Isaac U, Asuzu M, Maghsoud K and Ghazi-Khansari (2014).Effect of aqueous extract of Hibiscus sabdariffa on some biochemical parameters in alloxan-induced diabetic rats *Comparative Clinical Pathology* DOI 10.1007/s00580-014-1889-7

23. Pazdro and J.R.Burgess,(2010). The role of vitamin E and oxidative stress in diabetes complications, Mechanism of Ageing and Development, 131,276-286.

24. Peskin AV and Winterbourn, CC. (2017). "Assay of superoxide dismutase activity in a plate assay using WST-1," Free Radical Biology & Medicine. 103:188–191

25. Pryor WA and Stanley JP .(1975). Letter: A suggested mechanism for the production of malonaldehyde during the autoxidation of polyunsaturated fatty acids. Nonenzymatic production of prostaglandin endoperoxides during autoxidation. *J. Org. Chem.*, 40(24): 3615-3617.

26. Puepet FH, Ohwovoriole AE. (2008).Prevalence of risk factors for diabetes mellitus in a non-diabetic population in Jos, Nigeria. *Niger J Med*;17(1):71–74.

27. Robertson RP, Harmon J, Tran PO, Tanaka Y, and Takahashi H, (2003). Glucose toxicity in β cell type 2 diabetes, good radical gone bad and the gluthatione connection, *Diabetes*, 52, 581-587.

28. Sabir AA, Isezuo SA, Ohwovoriole AE. (2011).Dysglycaemia and its risk factors in an urban Fulani population of northern Nigeria. *West Afr J Med*;30(5):325–330.

29. Shrivastava A, Srivastava N, Kumar N. (2012). Phytochemical screening and study of analgesic activity of brinjal leaves. *Pharma Sci*.3:3028‑33.

30. Slaughter MR and O'Brien PJ.(2000). "Fully-automated spectrophotometric method for measurement of antioxidant activity of catalase," Clinical Biochemistry. 33(7)525–534.

31. Trease G, Evans W. (2002). *Pharmacognosy*, 15 ed. Harbcourt publishers Ltd., WB Saunders Company Ltd;.

32. Vives-Bauza C, Starkov A, and Garcia-Arumi E. (2007) "Measurements of the antioxidant enzyme activities of superoxide dismutase, catalase, and glutathione peroxidase," Methods in Cell Biology. 80:379–393.

33. Wasowicz W, Neve J, Peretz A.(1993). Optimized steps in fluorometric determination of thiobarbituric acid reactive substances in serum: importance of extraction pH and influence of sample preservation and storage.*ClinChem*.39: 2522 – 2526.

34. Whiting DR, Guariguata L, Weil C, Shaw J (2011). IDF diabetes atlas: Global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Research Clinical Practice*; 94:311-21.

35. World Health Assembly (2013). Follow-up to the Political Declaration of the High-level Meeting of the General Assembly on the Prevention and Control of Non-communicable Diseases. *Geneva*: World Health Organization.

36. Wright Jr. E, Scism-Bacon JL and Glass LC (2006) Oxidative stress in type2 diabetes: The role of fasting and postprandial glycaemia. *International Journal of Clinical Practice* 60: 308–314.

37. Bamanika SA, Bamanikar AA and AroraA .(2016).Study of serum urea and Creatinine in diabetic and non-diabetic patients in a tertiary teaching hospital. *Journal of Medical Research*. 2(1): 12-15