EVALUATION OF SOME BIOCHEMICAL 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 of diabetic male Wistar rats. 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 showed that the extract significantly improved diabetes induced decrease in Cl^- 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 showed 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 fatigue due to abnormal glucose metabolism. 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 glucose causing oxidative stress and worsening damage to the pancreatic β cells and other organs [Robertson etal., (2003); Pazdro and.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, cardiopathy and 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% [Nyenwe etal.,(2003): Puepet and Ohwovoriole (2008): Sabir etal.,(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. There is an increasing interest in research on medicinal plants with antidiabetic potential. *Hibiscus sabdariffa* is a medicinal plant grown as a yearly shrub in some Asian and African countries including Nigeria. 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 Ebong et 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 manual centrifuge. Then, 1 mL aliquots of serum were removed and used to perform the biochemical assay. In analysing biochemical parameters, commercial Labtest Diagnostic kits were used, with standard techniques based on enzymatic and colorimetric methods, spectrophotometry, 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); Wasowicz etal., (1993); Slaughter and O'Brien (2000);Vives-Bauza etal., (2007);Condezo-Hoyos etal., (2013); Peskin and Winterbourn,(2017)]

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 1-5.

Table 1: Blood glucose level of Wistar rats

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

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

Table 2: Serum lipid profile 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	TC (mmol/l)	TG (mmol/l)	LDL (mmol/l)	HDL (mmol/l)
Negative Control	2.88±0.16	1.12±0.07	1.77±0.12	1.62±0.07
Diabetic Control	3.08±0.04	1.26±0.03	1.86±0.12	1.58±0.11
250mg/kg	2.92±0.25	1.26±0.04	1.34±0.09[#]	2.10±0.17[#]
500mg/kg	2.76±0.07[#]	1.36±0.12	1.48±0.10[#]	2.02±0.11[#]

Table 3: 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)	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*	26.00±1.41
250mg/kg	132.00±0.84	4.24±0.22	66.00±1.58[#]	25.60±1.72
500mg/kg	135.80±1.42	4.58±0.12	67.00±1.00[#]	27.60±0.79

Table 4: Oxidative stress markers of Wistar rats after *Hibiscus sabdariffa* extract administration

Groups	GSH (µg/ml)	CAT (µ/g)	SOD (µ/ml)	MDA (µmol/ml)
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$^{\#}$	0.21±0.03	0.38±0.05$^{\#}$
500mg/kg	1.23±0.18	4.79±0.50$^{\#}$	0.26±0.02	0.27±0.05$^{\#}$

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

Table 5: Phytochemical analysis of aqueous calyx extract of *Hibiscus sabdariffa*

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 alloxan in rats that received no other form of drug treatment during the study (Ebong.,2008). Alloxan has 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-c after 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 level in 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 flavonoids are 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 their pharmacological 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.has been shown to be responsible for its range of pharmacological effects.

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