Evaluation of Selected Biochemical Indices of Diabetic Rats Treated with Methanol Leaf Extract of *Andropogon gayanus*

Abstract

The aim of this study was to evaluate biochemical changes in diabetic Wistar rats administered methanol leaf extract of *Andropogon gayanus*. Freshly harvested leaves of *A. gayanus*were processed into extract.Twenty-five adults male Wistar rats were divided into five groups of five rats per group.**Group I** was the normal controland was administered 2 mL of distilled water. **Group II**: was induced diabetes without treatment.**Group III:** was diabetic rats treated with 100 mg/kg of *A. gayanus* leaf extract. **Group IV:** was diabetic rats treated with 200 mg/kg of*A.gayanus* leaf extract. **Group V:**wasdiabetic rats treated with standard drugs. The activities of AST, ALT and ALP in diabetic groups treated withthe aforementionedextract were significantly ($P<0.05$) lower than those reported for the normal control. However, a contrary observation was made on the serum albumin and total protein.Total cholesterol (TC) and Triglycerides and Low-Density Lipoprotein reported for groups treated with extract were significantly ($P<0.05$) higher than those reported for the negativecontrol. However, a contrary observation was made on the High-Density Lipoprotein (HDL). Serum creatinine and urea reported for the negative control were significantly ($P<0.05$) higher than those reported groups administered leaf extract of *Andropogon gayanus*. In conclusion, it can be deduced from this study thathas influence on the indices evaluated which be traced to the biological activities of certain active ingredients inherent in them.

Keywords: Protein, Serum, Diabetes, *Andropogon gayanus*, Albumin

Introduction

Diabetes mellitus (DM) commonly referred to as diabetes is a renowned disorder of metabolism characterized by hyperglycemia, a physiologically abnormal conditionin which blood glucose level is markedly raised. Itresults from either impaired secretion of insulin or insulin action or both (ADA, 2014; ADA, 2018).Being one of the leading causes of morbidity and mortality globally, it is considered a major public health concern (Akter et al., 2014; Zeng et al, 2014). This is evident by the fact that an estimated 1 in 11 adults suffer from diabetes across the globe (IDF, 2017).Etiologically, diabetes is strongly associated with lipid disorder, kidney damage and susceptibility impaired liver functions (WHO, 2005; Philip et al. 2014).

The use of plant-based medication in the treatment of diseases dates back to prehistoric times and has progressed steadily through generations owing to its affordability, safety and ease of application.(Corbin, 1998).*Andropogon Gayanus*commonly known as gamba grass is a species of grass, native to most of the tropical and sub-tropical Savannas of Africa. It is a 4 meters (13ft) tall tree. The leaf is 30-60 cm long with a distinctive white midrib covered with soft hairs. It is a member of the *Poaceae* family. *Andropogon gayanus*has been used extensively in the treatment diverse human diseases across the continent of African owing to it rich medicinal values. Specifically, *A. gayanus* is employed in the treatment of cough, diarrhoa, bronchitis, postpartum pain and leg ulcer (Corbin, 1998).

Materials and Methods

Collection of plant Material

Fresh leaves of*Andropogon Gayanus*were obtained froma farm land in Afikpo North Local Government Area of Ebonyi State. The plant material was conveyed in a plastic bag to the herbarium unit of the Department of Agronomy, Michael Okpara University of Agriculture Umudike, Abia State South-East Nigeria where it was identified. The leaves were subsequently washed with clean tap water and afterwards air dried at room temperature. The dried leaves were ground into fine powder with the aid of an electric blender.

Extraction of Plant Material

The powderedplant sample (1000 g) was extracted using aqueous methanol by cold maceration with occasional shakingafter every 48 hours for 7 days. The solvent was allowed to evaporate to dryness at room temperature. The extract was then stored in a desiccator until needed for the study. Fresh solutions of the extract were prepared in distilled water for each study.

Animals

Adults male Wistar rats weighing 150-180 g were procured from the animal house of the Department of Science LaboratoryTechnology, AkanuIbiam Federal Polytechnic Unwana Afikpo. The rats were housed in well-ventilated cages under standard laboratory conditions.They were fed on standard laboratory animal feeds (Vital feed pellets) and water *ad libitum*. All experiments performed on the laboratory animals in this study was based on the Principles of laboratory animal care (NIH Publication, 1996).Acclimatization of the animals lasted for two weeks prior to the study.

Median Lethal Dose (LD 50%)

Median lethal dose of *Andropogon Gayanus* leafextract was determined in accordance with themethod described by Lorke (1983). The is characterized by two two phases. At the initial phase, three (3) groups of three rats were administered with extract at the doses of of 10, 100 and 1000 mg/kg body weightorally and observed for signs of toxicity for 24 hours. At the second phase, three groups of one ratper group wereadministered with three more specific doses of the extract.The LD_{50} value was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (0/1 and 1/1).

Induction of Diabetes

Induction of diabetes mellitus was performed by a single intraperitoneal injection of 60 mg/kg body weight of streptozotocin, dissolved in 0.1mol/L fresh cold citrate buffer, (pH 4.5) into rats that had been fasted for 24 h (Burcelin*et al.*, 1995). After 3 days, the blood sugar levels were determined with the aid of a glucometer. Rats with fasting blood glucose level more than 126mg/dl (11.1mmol/L) were considered diabetic and thus used for the study.

Animal Grouping

Group I: (Normal Control) animals were administered distilled water
Group II: Diabetes induced without treatment
Group III: Diabetes + 100 mg/kg of *Andropogon Gayanus*leaf extract
Group IV: Diabetes + 200 mg/kg of Andropogon*Gayanus*leaf extract
Group V: Diabetes + standard drug

Biochemical analysis

Sample preparation: To perform liver and kidney function tests, exactly 2 mL of blood introduced into the EDTA tube was centrifuged at 4,000 rpm for 15 min and the plasma obtained was stored for biochemical analysis.

Determination of Liver Enzyme Activity

Liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP)were determined by themodified Reitman and Frankel method for AST, ALP and ALT estimation(Reitman and Frankel, 1957).

Determination of Urea

Exactly 10 µL of sample was introduced into a tube holding 1000 µL of the working reagent. The contents of the tube were thoroughly mixed, incubated for 5 minutes at 37^{o}C(Kaplan, 1982). Blood urea concentration was determined with the aid of the formular below:

$$Urea\ conc\ (mg/dl) = (A\ Sample)/(Acal/STD) \times conc.cal/STD(mg/dl)$$

Determination of Serum Creatinine

Exactly 0.1 mL of sample was added into a tube holding 1.0 ml of working reagent and the content thoroughly mixed. After 30 seconds, the initial absorbance (A1) of the sample and standard were read. This was repeated after 2 minutes was read (Bartels and Bohmer, 1972). The following formular was employed to determine serum creatine level.

$$Creatinine\ conc.(mg/dl)\ \ (\Delta A\ sample)/\Delta Asample \times standard\ conc.(mg/dl)$$

Determination of Lipid profile

Serum total cholesterol and LDL-cholesterol were evaluated by Enzymatic and-point method (Unit mmol/L) (Kayamori, et al., 1979). Triglycerides concentration was determined in accordance with the method described by Trinder (Trinder, 1969) while the procedure of Wieland and Siedel(1981) was employed to determine of HDL-cholesterol.

Estimation of serum total protein

The biuret method was employed to determine the serum protein concentration(George, 2009). The principle underlying this reaction is that serum proteins react with copper sulphate in sodium hydroxide to form a violet biuret complex. The intensity of the violet colour measured with the spectrophotometer is proportional to the concentration of protein (Bjorsten et al, 2007).

Estimation of serum albumin

The dye-binding technique was employed to determine the serum albumin content. It utilizes the ability of albumin to form a stable complex with bromocresol green dye (George, 2009). The absorbance of the sample and that of the standard was measured against the reagent blank at 546 nm, and temperature of 37°C. The tubes and their contents were mixed and incubated for 90 minutes at 37°C.

Data Analysis

Data obtained were expressed as Mean ± Standard Deviation using SPSS (Ver. 23). Data were analysed using one way Analysis of Variance (ANOVA). Differences in mean were compared using Turkey Test. *p-values* less than 0.05 was considered statistically significant.

Table 1: Serum Hepatomarkersin Diabetic Rats treated with *Andropogon gayanus Leaf Extract*

Treatment group	AST(U/I)	ALT(U/I)	ALP(U/I)	Total protein(g/l)	Albumin (g/l)
Normal Control	19.98±0.86^{a}	37.82±2.04^{a}	58.92±2.14^{a}	70.21±2.24^{c}	39.28±3.62^{c}
Negative Control	25.73±2.12^{d}	46.56±2.74^{c}	85.42±2.82^{d}	41.20±2.20^{a}	26.52±2.25^{a}
DE+AGLE	23.01±2.16^{c}	43.13±2.64^{bc}	69.98±3.02^{c}	65.20±3.23^{b}	33.02±2.32^{b}
DE+AGLE	23.12±0.78^{c}	41.12±2.16^{b}	64.58±2.65^{bc}	66.34±4.10^{c}	38.42±3.32^{c}
DE $_{+}$STD DRUG	22.09±2.26^{b}	41.76±0.98^{b}	62.24±3.34^{b}	74.34±2.40^{d}	42.30±0.37^{d}

Results are expressed as mean ± standard deviation. Values with the same superscript in a column are not significantly different at ($P<0.05$).

Table 2: Lipid Profiles in Diabetic Rats treated with *Andropogon gayanus Leaf Extract*

Treatment group	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Normal Control	203.90±2.12^{a}	62.22±4.67^{b}	75.30±4.30^{e}	126.54±3.06^{a}
Negative Control	260.32±2.30^{c}	100.05±09.23^{e}	42.41±2.34^{a}	205.99±2.32^{c}
DE+ $AGLE_{100\ mg}$	225.34±2.13^{b}	72.20±2.03^{c}	62.30±3.02^{b}	140.58±2.40^{b}
DE+ $AGLE_{200\ mg}$	227.58±2.32^{bc}	76.02±4.01^{d}	63.07±3.70^{c}	140.74±1.29^{b}
DE $_{+}$STD DRUG	203.0.9±2.08^{a}	60.56±2.05^{a}	65.32±5.40^{d}	126.08±1.85^{a}

Results are expressed as mean ± standard deviation. Values with the same superscript in a column are not significantly different at ($P<0.05$).

Table 3: Serum Renal Function Markers in Diabetic Rats treated with *Andropogon Gayanus Leaf Extract*

Treatment group	Urea	Creatinine
Normal Control	2.03 ± 0.03^{a}	0.65 ± 0.03^{a}
Negative Control	4.51 ± 0.32^{c}	2.71 ± 0.06^{c}
DE+AGLE$_{100\ mg/kg}$	3.52 ± 1.02^{bc}	1.32 ± 0.04^{b}
DE+AGLE$_{200\ mg/kg}$	3.41 ± 0.20^{b}	1.43 ± 0.02^{bc}
DE +STD	3.26 ± 0.35^{b}	1.32 ± 0.23^{b}

Results are expressed as mean ± standard deviation. Values with the same superscript in a column are not significantly different at ($P<0.05$).

Results and Discussions

Research has shown that correlation between diabetes mellitus and hepatic dysfunction abound. Table 1 shows the serum hepatomarkers in diabetic rats treated with methanol leaf extract of *Andropogon gayanus*,indicating that the activity of aspartate aminotransferase (AST) reported for the diabetic control was significantly ($P<0.05$) higher than that reported for the normal control. However, the increased activity of the said enzyme observed after induction of diabetes was significantly ($P<0.05$) reduced following oral administration of extract though to levels which were significantly ($P<0.05$) lower than that reported for the normal control but lower than that reported for the group administered standard drug. Similar observation was made on Alkaline phosphatase (ALP) and Alanine aminotransferase (ALT). Itis worthy to note that the ALP and ALT activities reported for the groups administered extract were not significantly ($P<0.05$) different from those reported the group administered standard drug. The ability of the said plant part to reduce the activity of liver enzymes could be attributed to the presence of certain medicinal phytochemicals which had reportedly demonstrated hepatoprotective as well as antioxidant potentials (Zandam et al. 2020). This is consistent with the finding of Oraobosi and Elendu (2021) which showed that oral administration of *A. gayanus*extract reduced the activity of liver enzymes in paracetamol induced liver toxicity.Serum total protein reported for the diabetic control was significantly ($P<0.05$) lower than that reported for the normal control. However, it was observed to increase in a dose dependent manner in groups administered extract to levels which were significantly ($P<0.05$) lower than that reported for the group administeredstandard drug.Diabetes mellitus has been known to be associated with lipid disorder. Table 2 Table shows the lipid profile of diabetic rats treated with methanol leaf extract of *Andropogon gayanus* indicating that Total cholesterol observed following induction of

diabetes was significantly (P<0.05) high but was reduced following treatment with extract to levels which were significantly (P<0.05) higher than that reported for group administered standard drug which in turn was not significantly (P>0.05) different from that of the normal control. Triacylglycerol was significantly (P<0.05) raised following diabetes induction, an observation which was reduced in a dose dependent manner to levels which were significantly (P<0.05) lower than that reported for the normal control which in turn was significantly (P<0.05) higher than that reported for the group administered standard drug. High Density Lipoprotein was reportedly low following diabetes induction. However, this was raised in groups administered extract in a dose dependent manner. HDL was significantly (P<0.05) lower in groups administered extract than that reported for the group administered standard drug which in turn was significantly (P<0.05) lower than that reported for the normal control. Diabetes induction significantly (P<0.05) raised the level of Low-DensityLipoprotein. However, it was observed to be reduced following oral administration of extract. The LDL level reported for the extract group was significantly (P<0.05) higher than that reported for the group administered standard drug which was not significantly (P<0.05) different from that reported for the normal control.Studies have shown that the natural active ingredients in some plants have unique advantages in treating dyslipidaemia (Ren et al. 2019). Furthermore, many studies have emphasized the hypolipidemic benefits of phytochemicals, which are multi-component, multi-targeted, and have relatively low toxic effects (Islam et al. 2021).Therefore, it is not out of place to infer that the phytochemicals inherent in the leafof*Andropogon gayanus* may have contributedto its ability to restore a healthy lipid profile in diabetic rats. Sustained hyperglycaemia is the main cause of the changes in kidney function in diabetes mellitus. Hyperglycaemia leads to the increased formation of advanced glycation end-products (AGEs),

oxidative stress, activation of the polyol pathway and hexosamine flux, causing inflammation and renal damage (Wolf and Ziyadeh, 2007).Creatinine and urea are the most frequently determined clinical indices for estimating renal function (Mitchell and Kline, 2006).Table 3 shows the serum renal function markers in diabetic rats treated with methanol leaf extract of *Andropogon gayanus* indicating that diabetes induction significantly ($P<0.05$) raised serum urea and creatinine level. However, after extract had been administered, a decrease in the aforementioned markers which was significantly ($P<0.05$) higher than the value reported for the normal control was observed. This is consistent with the finding of Seba et al. (2022) which showed that methanol extract of *Bambusaarundinacea* a member of the *Poaceae* family to which *Andropogon gayanus* belongs reduced seduced the level of urea and creatinine in rat.

Conclusion

It can be concluded that has influence on the indices evaluated which be traced to the biological activities of certain active ingredients inherent in them.Andropogon gayanus has been used extensively in the treatment diverse human diseases across the continent of African owing to it rich medicinal values.

References

[1] American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2014;37: S81–90.

[2] American Diabetes Association. Microvascular complications and foot care: Standards of medical care in diabetes—2018. Diabetes Care. 2018;41: S105–18.

[3] Akter S, Rahman MM, Abe SK, Sultana P. Prevalence of diabetes and prediabetes and their risk factors among Bangladeshi adults: a nationwide survey. *Bull World Health Organ*. 2014; 92:204-213A.

[4] Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat Rev Endocrinol*. 2018;14(2):88.

[5] IDF. International Diabetes Federation. IDF Diabetes Atlas — 8th Edition. Diabetes Atlas. https ://diabetesat las.org/resources/2017-atlas.html. 2017. Accessed March 2019.

[6]

[7] Philip R, Mathias M, Kumari SN, Gowda DK, Shetty JK. Evalation of relationship between markers of liver function and the onset of type 2 diabetes. *Nitte Univ J Health Sci*. 2014;4(2):90.

[8] Corbin WL. Herbs as Medicines. *Archives of Internal Medicine*. 1998; 158: 2192-2199.

[9] NIH (1993). NIH Consensus Development Panel on Impotence.*JAMA*270, 83-90.

[10] Lorke, D. (1983). A new approach to practical acute toxicity testing. Archive Toxicology. **54**:275-287.

[11] Burcelin, R., Eddouks, M., Maury, J., Kande, J., Assan, R. and Girard, J. (1995). Excessive glucose production, rather than Insulin resistance, account for hyperglycemia in recent onset streptozotocin-diabetic rats. Diabetologia, **35**:283-290.

[12]Reitman.S, and Frankel.S. Modified Reitman and Frankel method of AST and ALT estimation. *Amer.J.Clin.pathol*,1957;28:56-63.

[13] Kaplan, A and Teng, L.L. (1982). Selected Methods of Clinical Chemistry. In W.R. Faulkner &Mietes, S (Eds.), (pp 357-363). AACC, Washington.

[14] Bartels, H and Bohmer, M. (1972) Clinical Chemistry. Acta, 37: 193.

[15] H Wieland, D Siedel, *Artzl. Lab*., **1981**, 27, 141-144.

[16] Bjorston,A.R, Crankshaw,D.P, Morgan,D.J,Prideaux,P.R (2007). Clinical Chemistry. Department of surgery, Royal Malbourne Hospital, University of Melbourne,Victoria, 3050.

[17] George R. K. (2009). Biochemistry Laboratory. Philadelphia www.jbc.org

[18] Zandam SU, Nazifi AB, Odoma S, Zezi AU. Anti-nociceptive and anti-inflammatory activities of methanol root extract of Andropogon gayanusKunth (Poaceace) in rodents. Afr J Pharmacol Ther. 2020; 9(1):27-33.

[19] Michael I. Oraebosi and Melford U. Elendu. Hepatoprotective Effect of Andropogon gayanus against Paracetamol- and CarbonTetrachloride-Induced Liver Toxicity in Rats, Tropical Journal of Natural Product Research. Trop J Nat Prod Res, January 2021; 5(1):188-193

[20] Ren, J.; Fu, L.; Nile, S.H.; Zhang, J.; Kai, G.Y. Salvia miltiorrhiza in Treating Cardiovascular Diseases: A Review on Its Pharmacological and Clinical Applications. Front. Pharmacol. 2019, 10, 753.

[21] Ul Islam, S.; Ahmed, M.B.; Ahsan, H.; Lee, Y.S. Recent Molecular Mechanisms and Beneficial Effects of Phytochemicals and Plant-Based Whole Foods in Reducing LDL-C and Preventing Cardiovascular Disease. Antioxidants 2021, 10, 784.

[22] Wolf G, Ziyadeh FN. Cellular and molecular mechanisms of proteinuria in diabetic nephropathy. Nephron Physiol 2007; 106: 26–31.

[23] Nephroprotective Potential of a Standardized Extract of *Bambusaarundinacea*: In Vitro and In Vivo Studies Saba Muzher Iqbal, Liaqat Hussain, Musaddique Hussain, Haseeb Akram,

Muhammad Asif, Ayesha Jamshed, Ammara Saleem, and Rida Siddique. This: ACS Omega 2022, 7, 18159−18167.