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Determination of Genomic DNA Concentration and Purity in Diabetic Rats (*Rattus norvegicus*) Treated with Aqueous Extract of *Saccharum officinarum* Stem Bark.

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ABSTRACT

Reliable measurement of genomic DNA quantity and quality is a fundamental variable in diabetic research. The aim of the research was to evaluate the quantity and quality of dsDNA in the liver of type 2 induced diabetic rats after treatment with aqueous extract of *Saccharum officinarum* stem bark.

25 apparently healthy young male rats (5-6 weeks old; weighing between 40-60g) were procured for the study. Diabetes was induced via high fat diet feeding for 12 weeks followed by STZ injection (40 mg/kg bw). 15 rats were confirmed diabetic after induction and used for the study. An additional group (normal control) of 5 rats was administered distilled water 1 ml/kg bw. A diabetic group was administered normal saline 1 ml/kg bw, another group was treated with metformin 500 mg/kg bw, while the last group was treated with 500 mg/kg bw of the extract. Treatments lasted for 14 days via the oral route. Liver tissue samples were collected after anaesthesia with ketamine via dissection and preserved in liquefied nitrogen for genomic studies while blood glucose level was checked in serum of rats with a test kit. Genomic dsDNA was extracted from homogenized liver tissues. The yield and purity was estimated via nanodrop spectrophotometry at an absorbance of 260nm and an absorbance ratio A260/280. The Results showed decrease in the fasting blood glucose level in the serum of the treated rats which was significant ($p<0.05$). Likewise, dsDNA yield and purity was increased in the treated rats and it was significant ($p<0.05$). In conclusion the aqueous extract of *Saccharum officinarum* stem bark was able to ameliorate the diabetic condition in the rats by causing an increase in the genomic DNA yield and purity in addition to attenuating hyperglycaemia in the rats.

Keywords: Diabetes mellitus, DNA yield, DNA purity, Nanodrop spectrophotometry

INTRODUCTION

Diabetes mellitus is a devastating illness emanating from the pancreas with significant morbidity and mortality [1]. It's estimated that 25% of the global population is already affected by this disease with 80% of the people living in African and Asian countries [2, 3]. In Nigeria, the prevalence rate of this disease has more than doubled over a decade ago from a rate of 2.2% to a range between 9-15% [4]. [5] have found a prevalence rate of 6.8% in adults older than 40 years. Earlier, [6] have found estimated prevalence rate of 5.7-7.7% for females and males in Port-Harcourt, in the southern part of Nigeria. [7] and [8] in a survey of diabetes mellitus in Makarfi and Dakaci villages both sub-urban communities in Zaria local government area of Kaduna state have reported prevalence rates of 1.6% and 2.0% respectively. [9] in a study on the prevalence of diabetes mellitus in Nigeria reported type 2 diabetes mellitus to be the most common type of diabetes affecting Nigerians accounting for about 90% of cases.

In modern medicine no satisfactory effective therapy is still available to cure diabetes mellitus [10]. The conventional chemical drugs and even insulin therapy used for the management of diabetes mellitus still present drawbacks like insulin resistance [11], anorexia nervosa, brain atrophy and fatty liver [12] after chronic treatment. Recently, the search for appropriate hypoglycaemic agents has been focused on plants used in traditional medicine partly because of leads provided by traditional medicine to natural products that may be better treatments than currently used drugs [13], because these are more harmonious with the biological system [14].

Herbs have usually little or no toxicity during long-term administration, the cost is significantly low and are relatively available at large scale [15].

Few of the plants used for the treatment of diabetes mellitus have received scientific or medical scrutiny and even the World Health Organization expert committee on diabetes recommended that this area warrants further attention [16, 17].

Saccharum officinarum is a perennial crop that belongs to the family Poaceae and grows in warm temperate and

tropical regions. It is indigenous to Asia and Africa. It is also one of the regularly consumed plant resources as food materials.

Earlier study have shown extracts of *Saccharum officinarum* stalk and leaves to have antihyperglyaemic activity in diabetic rats [18, 19].

MATERIALS AND METHODS

Plant material

Stem barks of *Saccharum officinarum* (sugarcane) were obtained from farms in Giwa local government area of Kaduna state and authenticated at the Herbarium unit of Biological Science Department, Ahmadu Bello University, Zaria, and issued a voucher number 1086.

Experimental animals

25 young (5-6 weeks old) male Wister rats (weighing 50-70g) were acquired for the study. The rats were kept in cages and allowed access to water and food (rodent diet) *ad libitum*. All the rats were allowed to acclimatize to the laboratory conditions for two weeks. Rats were treated humanely and in accordance with the guidelines stipulated by the Ahmadu Bello University Committee on the use and care of laboratory animals for research purposes.

Plant preparation and extraction

The fresh plant of *Saccharum officinarum* purchased was washed with water to remove dirt/contaminants. The bark was peeled off and air dried in the laboratory. Dried bark was pounded into a coarse powder using pestle and mortar. 2 liters of distilled water was added to 500g of powdered bark material and stirred in a conical flask until evenly mixed. The mixture was filtered using muslin cloth followed by Whatman filter paper. The resultant filtrate was placed on a steam bath (50^0C) until all the moisture evaporated.

High fat diet (HFD) feeding and weight measurement

High fat diet containing 50g animal derived fat and 100g of grower's mash was fed to the rats morning and evening for 12 weeks with weekly body weight measurements with the aid of a top-loading weighing scale (KERRO BL20001, Taiwan).

Type 2 diabetes mellitus induction

This was done with streptozotocin (STZ) injection 40 mg/kg body weight (Zayo Sigma, Nigeria) at a P^H of 4.5 given intraperitoneally. Hyperglycaemia was analyzed via fasting blood glucose level from blood collected from the tail of rats 1 week after induction with an AccuCheck Active glucometer (Roche®). Rats with $\geqslant$ 200 mg/dl blood glucose level were used for the research.

Experimental design

Table 1: Grouping and treatment

GROUPS	DOSE
1 (Normal control)	1 mg/kg body weight of distilled water.
2 (diabetic, no treatment)	1 mg/kg body weight of normal saline.
3 (diabetic + metformin)	500 mg/kg body weight of metformin.
4 (diabetic + extract)	500 mg/kg body weight of extract.

Duration of treatment: 14 days.

Route of administration: oral gavage.

Sacrifice and sample collection

Rats were fasted overnight, anaesthesia was done with ketamine hydrochloride (50 mg/kg body weight), intraperitoneally. Blood was collected via cardiac puncture into plain tubes, allowed to clot and centrifuged at 1656 rev/minute for 5 minutes to obtain the serum. Blood glucose level was again measured in the serum of all rats with the aid of a glucose test kit (AGGAPPE).

Liver tissues were collected via dissection and preserved in liquefied nitrogen for genomic studies.

Preparation of tissue for the genomic studies

Sampling

Appropriately preserved liver tissues were randomly selected and used for genomic DNA extraction.

Genomic DNA extraction

5mg of slice liver tissue was homogenized and placed into a 2mL micro tube, 20µL lysis buffer and 100µL PBS were added and mixed thoroughly by vortexing. The mixture was incubated at 55^{0C} overnight on rotatory shaker to completely lyse and dissolve the tissue. 200µL absolute ethanol was added and mixed thoroughly again by vortexing. The mixture was pipetted into a DNeasy Mini spin column placed in a 2mL collection tube and centrifuged at 8000 rpm for 1 minute. The flow-through and collection tube were discarded while the spin column was placed in a new 2mL collection tube and 500µL of wash buffer 1 (AW1) was added and centrifuged at 8000 rpm for 1 minute.

The flow through and the collection tube were discarded and the spin column placed in a new 2mL collection tube. 500uL of wash buffer 2 (AW2) was added into the tube and centrifuged for 3 minutes at 14000 rpm. The flow-through and collection tube were discarded. The spin column was finally centrifuged to dryness at 14000 rpm for 30 seconds. The extracted dsDNA was eluted by adding 100 µL of elution buffer.

Absorbance method

A nanodrop (1µL) of extracted dsDNA was dropped onto the measurement point of a spectrophotometer equipped with a UV lamp (ND-1000 Thermo Fischer Scientific). DNA yield was estimated by measuring absorbance of the dsDNA sample at a wavelength of 260nm. DNA purity was estimated by calculating the absorbance ratio (A260/280). Plots of the spectral pattern of the absorbance were displayed on a computer monitor connected to the spectrophotometer.

RESULTS

a) Body weight changes

Figure 1 shows the means of body weights changes of control and experimental rats for the 12 weeks period. The result indicated a significant increase ($p<0.05$) in the body weights of the experimental groups when compared to the normal control during the 12 weeks period of high fat diet feeding.

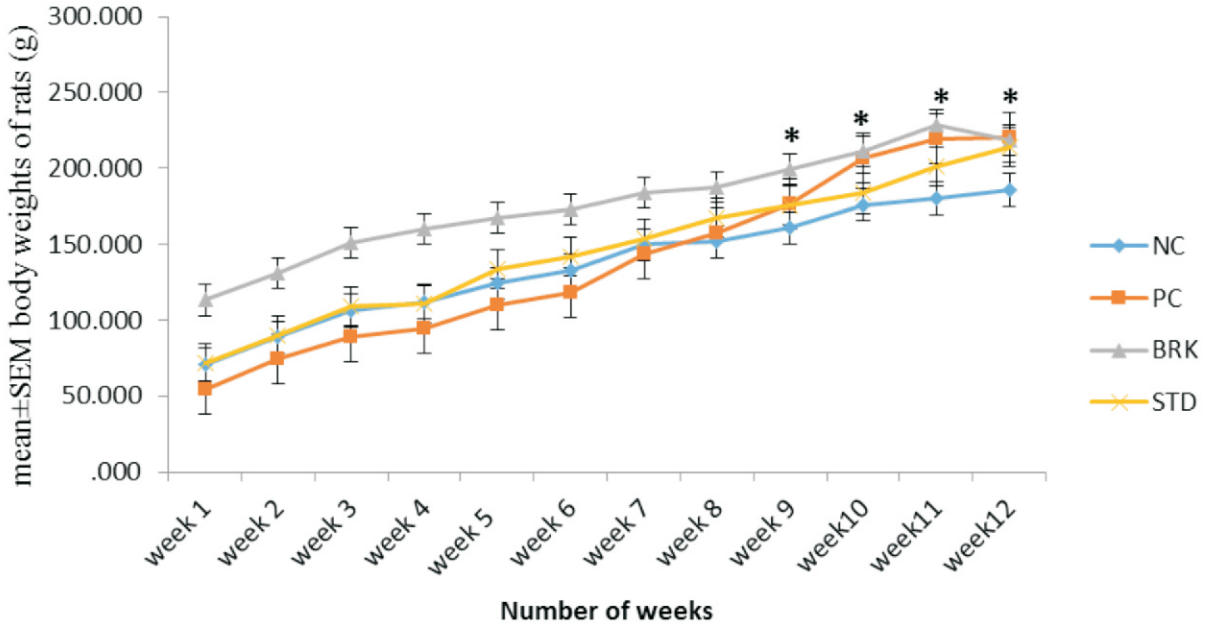

Figure 1: Means of body weights of control and experimental rats for a 12 week period. *indicates statistical significant difference between normal control (NC) and other experimental groups ($p<0.05$). PC-positive control (diabetic), BRK-stem bark, STD-standard drug (metformin).

b) Serum fasting blood glucose

Table 2 showed the ameliorative effect of metformin and aqueous extract of *Saccharum officinarum* stem bark on the serum blood glucose in all experimental groups. The result indicated a significant decrease ($p<0.05$) in the serum fasting fasting blood glucose of the diabetic rats when compared to the normal control.

Table 2: Means of serum fasting blood glucose (FBG) mg/dl of control and experimental groups.

PARAMETER	GROUP 1 (NC)	GROUP 2 (PC)	GROUP 3 (STD)	GROUP 4 (BRK)	p
FBG (mg/dl)	61.77±9.91	112.59±15.37*	35.61±10.22	49.85±8.71	0.02

*indicates statistical significant difference when compared to normal the control ($p<0.05$).
Key: NC-normal control); PC-positive/diabetic control; STD-standard drug (metformin); BRK-stem bark (extract).

c) Genomic dsDNA estimation

The concentration and purity of genomic dsDNA showed that there was an increase in the DNA yield and purity in the liver of the treatment groups when compared to the normal control. This increases were significant ($p<0.01$).

Table 3: Means of genomic dsDNA yield and purity in the control and experimental groups.

Groups	Sample type	Concentration (ng/µl)	DNA purity (A260/280)	P
1. Negative control	dsDNA	336.48±2.81	2.09	0.01
2. Positive control	dsDNA	36.80±1.81*	0.86	
3. Diabetic (metformin)	dsDNA	434.56±14.08*	2.10	
4. Diabetic (extract)	dsDNA	415.29±6.96*	2.07	

* Indicates statistical significant difference when compared to the normal control ($p < 0.05$).
Pure DNA has a ratio of 1.8-2.0 [20]

DISCUSSION

The present study described a spectrophotometric method for estimating genomic DNA yield and purity in diabetic rats after treatment with aqueous extract of *Saccharum officinarum* stem bark.

High fat diet feeding for a period of 12 weeks resulted in the induction of obesity an important marker in the pathogenesis of type 2 diabetes mellitus [21]. This caused a corresponding increase in the body weights of the rats for the period of the feeding.

[18, 19] had earlier reported stalk and leaves of *Saccharum officinarum* to have the potential to reverse hyperglycaemia in experimental models. In agreement our study after 14 days intervention with the aqueous extract of *Saccharum officinarum* stem bark showed amelioration of hyperglycaemia in the diabetic rats. This may mean that the extract may contain bio-molecule(s) that may have caused an increase in the sensitivity of the insulin receptor in target organs such as liver and muscle and thus a reversal of hyperglycaemia.

Based on the genomic analysis, we found a significant decrease in the concentration and purity of genomic DNA in the diabetic untreated group. However, groups that were exposed to the intervention therapy had a significant increase in genomic DNA content and purity when compared to the normal control. We suppose that an increase in the DNA concentration and purity portrayed the ameliorative potential(s) of the extract on type 2 diabetes.

CONCLUSION

From the results showed in our study, we concluded that, the aqueous extract of *Saccharum officinarum* stem bark had anti-diabetic potentials in type 2 induced diabetic rats by improving genomic DNA quantity and purity in the liver.

Furthermore, while these antidiabetic effects in the rat models may reflect similar effects in humans, it does not necessarily mean clinical improvement on the overall, as such it is still necessary to confirm the present and subsequent findings in humans [22].

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