



Regeneration of Pancreas B-Cells in Alloxan-Induced Diabetes Rats Treated with Aqueous Seed Extract of *Syzygium aromaticum*: A Preliminary Study

Azeemat T. Abdulazeez¹, Kehinde H. Bello¹, Mutiu A. Alabi^{2*}, Fatai A. Kareem³ and Janet F. Adeegbe⁴

¹Biological Sciences Department, Faculty of Natural and Applied Sciences, Al-Hikmah University, Ilorin 240243, Nigeria.

²Medical Biochemistry and Pharmacology Department, College of Pure and Applied Sciences, Kwara State University, Malete 241104, Nigeria.

³Department of Pharmaceutical Science, School of Science and Technology, Gateway Polytechnic, Saapade 121104, Nigeria.

⁴Bioresources Development Centre, National Biotechnology Development Agency, Ogbomosho 210222, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors ATA, KHB and MAA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MAA FAK and JFA managed the analyses of the study. Author MAA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The present study was conducted to investigate the preventive/curative effect of aqueous seed extract *Syzygium aromaticum* in alloxan-induced diabetes in rats.

Study Design: Twenty Wistar rats were randomly divided into four groups. The first group served as a control (received 1 ml of normal saline). The second group served as Diabetic control (received 1 ml of normal saline). The third group served as test 1 (pre-treated with 500 mg/kg body weight

(b.w.) of aqueous *Syzygium aromaticum* seeds extract for seven days prior to diabetic induction and post-treated with aqueous seeds extract of *Syzygium aromaticum* after diabetic induction) and the fourth group served as test 2 (post-treated with 500 mg/kg b.w. of aqueous seed extract of *Syzygium aromaticum* after diabetic induction) in a daily oral dose for 14 days.

Results: Treatment of rats with aqueous seed extract of *Syzygium aromaticum* in a daily dose of 500 mg/kg significantly mitigates the induced changes in the glucose and lipid profile parameters. Histopathological examination of the pancreas showed the destruction of β -cells in the diabetic control group and recovery of damaged tissues when treated with 500 mg/kg b.w. of aqueous seeds extract of *Syzygium aromaticum*.

Conclusion: The present study suggests that aqueous seed extract *S. aromaticum* seeds extract at a dose of 500 mg/kg body weight brings about regeneration of the B-cells of the pancreas and significant beneficial effects in various physiological parameters in alloxan-induced diabetes.

Keywords: *Syzygium aromaticum*; diabetes; alloxan; blood glucose; pancreas.

1. INTRODUCTION

The consumption of a variety of local herbs and vegetables by man is believed to contribute significantly to the improvement of human health, in terms of prevention, and or cure of diseases because plants have long served as a useful and natural source of therapeutic agents [1-3]. Diabetes is a common and very prevalent metabolic disease condition in which there is an abnormal high level of blood sugar that culminates in diabetic complications affecting human and animals in both developed and developing countries [4-7]. An estimate of 25% of the world population is affected by this disease that manifests in various forms [8,9]. An individual is diabetic when his/her blood sugar level is above 180 mg/dl [10,11]. The effect of diabetes includes long term damage, dysfunction, and failure of various organs. Diabetes may present with characteristic symptoms such as thirst, polyuria, blurring of vision and weight loss [7,12].

Cloves are the aromatic flower buds of a tree in the family Myrtaceae, *Syzygium aromaticum* [13]. They are used in Indian Ayurvedic medicine, Chinese medicine, and western medicine herbalism as a carminative to improve peristalsis. Cloves are also said to be a natural anthelmintic [14,15]. The essential oil is used in aromatherapy when stimulation and warming are needed, especially for digestive tract. Topical application over stomach or abdomen are said to warm the digestive tract. Applied to a cavity in a decayed tooth, it also relieves toothache [16]. *Syzygium aromaticum* (Cloves) is an angiosperm plant, native of the Indian subcontinent, where its various parts have been utilized throughout history as food and medicine [13]. Dietary consumption of its part is therein promoted

strategy of personal health preservation and self-medication in various diseases.

Syzygium aromaticum is an angiosperm plant, native of Indian subcontinent, where its various parts have been utilized throughout history as food and medicine. Dietary consumption of its part is therein promoted as a strategy of personal health preservation and self-medication in various diseases. This study intent to corroborate the existing evidence of the plants' medicinal uses in the treatment and management of diabetes. The objective of the study is to evaluate the preventive role of aqueous seeds extract of *Syzygium aromaticum* on alloxan-induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagent

Alloxan monohydrate (powder) was a product of Sigma Chemical Company, Pooled, England. Cholesterol and triglycerides assay kits were products of Randox Laboratories, UK. All other chemicals and reagents are of analytical grade.

2.2 Plant Material

Dried seeds of *Syzygium aromaticum* were obtained from the Emir's Market, Ilorin, Kwara State. The plant was identified and authenticated by the curator at Department of Plant Biology of the University of Ilorin, Kwara State. A voucher specimen with voucher number (UIH 001/1107) of the plant was deposited in the University Herbarium.

2.3 Experimental Animals

Healthy male rats (*Rattus norvegicus*) with average body weight of 109 g were obtained

from a commercial breeder in Tanke, Ilorin, Kwara State, Nigeria and were housed in the animal house of the Biochemistry Unit, Department of Biological Sciences, Al-Hikmah University Ilorin, Kwara State, Nigeria. The rats were acclimatized for a period of seven days before the beginning of the experiment [17].

2.4 Sample Preparation

Hundred grams of *Syzygium aromaticum* was macerated in 100 ml of distilled water at room temperature for 24 hours. The resulting extract was filtered using muslin cloth and the resulting filtrate was concentrated using rotary evaporator under reduced pressure to give a semi solid residue which was then air dried.

2.5 Induction of Diabetes

Diabetes was induced according to the principle of Carvalhol et al. [18]. A single intraperitoneal injection of freshly prepared alloxan monohydrate at dose of 150 mg/kg in normal saline solution to a group of overnight fasted rat. After three days of alloxan administration, animals with blood glucose level of 200 mg/dl and above were considered diabetic.

2.6 Experimental Design

Rats were randomly grouped into four of five rats in each group; Group 1 (normal control) - received 1 ml of normal saline; Group 2 (Diabetic control) - received alloxan monohydrate at dose of 150 mg/kg in normal saline solution; Group 3 (Diabetic + extract) - pre-treated with 500 mg/kg b.w. of aqueous *Syzygium aromaticum* seven days prior to diabetic induction and post treated with *Syzygium aromaticum* after diabetic induction; and Group 4 (Post treated Diabetic) - post treated with 500 mg/kg b.w. of aqueous *Syzygium aromaticum* after diabetic induction. The extract at 500 mg/kg b.w. was chosen following the report of Sharma and Gupta [19]. The experiment lasted for 21 days after which the animal was sacrificed and blood was collected by jugular puncture under light anaesthesia. The blood was transferred into plain sample bottles.

2.7 Blood Glucose Measurement

The animals were fasted overnight to measure blood glucose level. Blood was collected from the animals via a drop of blood on the strip of the "on-call" glucometer by cutting about 1 mm of the

tail's end of the rats and result was expressed as mg/dl.

2.8 Biochemical Analysis

The lipid profile analysis done include total cholesterol (TC) and Triglycerides (TG) and was determined by method of Trinider [20] using enzymatic colorimetric diagnostic kits.

2.9 Histopathological Analysis

The pancreas was removed after sacrificing, wiped clean with tissue paper and stored in a container containing 10% formalin solution. The specimen was then dehydrated in ascending grades of ethanol, cleared in xylene, and processed to paraffin blocks, sectioned (Sum thick) and stained with haematoxylin and foin stain. It was examined using light microscopy for demonstration of pathological changes.

2.10 Statistical Analysis

The data were analysed using SPSS version 22.0 and the values were expressed as Mean \pm SEM (Standard Error of Mean). The means of the groups were compared using one-way ANOVA (Analysis of Variance) and level of significance was determined using Duncan Multiple Range Test (DMRT) AT $P < 0.05$.

3. RESULTS

The effect of aqueous extract of *Syzygium aromaticum* on body weight: The body weight of the rats among the groups were the same at the initial day and at the final day, respectively. Though, there was an insignificant difference when the weight of the final day was compared with that of the initial day in all the groups Table 1.

The effect of aqueous extract of *Syzygium aromaticum* on blood glucose: There was a significant increase of the blood glucose level in the diabetic control and test groups on the 3rd day after induction when compared with the control. The level was however decreased on the 7th day and was restored at the 14th day in both the pre-treated/post-treated diabetic and post-treated diabetic groups (Table 2).

The effect of aqueous extract of *Syzygium aromaticum* on lipid profile: Significant increase was observed in total cholesterol and triglyceride levels of the diabetic control group and a significant increase was observed in

triglyceride level of the pre-treated/post-treated diabetic while a significant decrease was observed in total cholesterol and triglyceride levels of the post-treated diabetic groups when compared with the control group Fig. 1.

Histopathological Analysis: The photomicrograph of the pancreas isolated from the animals showed the destruction of B-cells in the diabetic control group while there was recovery of the damaged tissues in the pre-treated/post-treated diabetic and post-treated diabetic groups Fig. 2.

4. DISCUSSION

Disturbances in glucose metabolism altered lipid levels and oxidative stress are important risk

factors for diabetes, cardiovascular, oncologic and many other diseases [21]. In diabetic condition, elevated blood glucose reduced body weight; polyuria and polyphagia are commonly observed. Alloxan induces “chemical diabetes” in a wide variety of animal species by damaging the insulin secreting pancreatic B -cell, resulting in a decrease in endogenous insulin release [22]. Numerous studies demonstrated that a variety of plant extracts effectively lowered the glucose level in alloxan-induced diabetic animals [23]. In this present study, induction of diabetes by alloxan produced increased in blood glucose levels. This may be due to insulin deficiency or resistance state in diabetic control rats [24].

Table 1. Effect of Aqueous extract of *Syzygium aromaticum* on body weight (g)

Group	Initial Day	Final Day
Control	96.0±6.38 ^a	118.6±8.62 ^a
Diabetic control	96.8±5.89 ^a	111.8±11.03 ^a
Pre-treated and Post-treated diabetic group	95.6±3.14 ^a	116.4±8.50 ^a
Post-treated diabetic group	96.4±3.97 ^a	113.0±1.87 ^a

Values are mean weight ± SEM (n=5). Different letters along the column and across the row indicate significant difference at $p < 0.05$

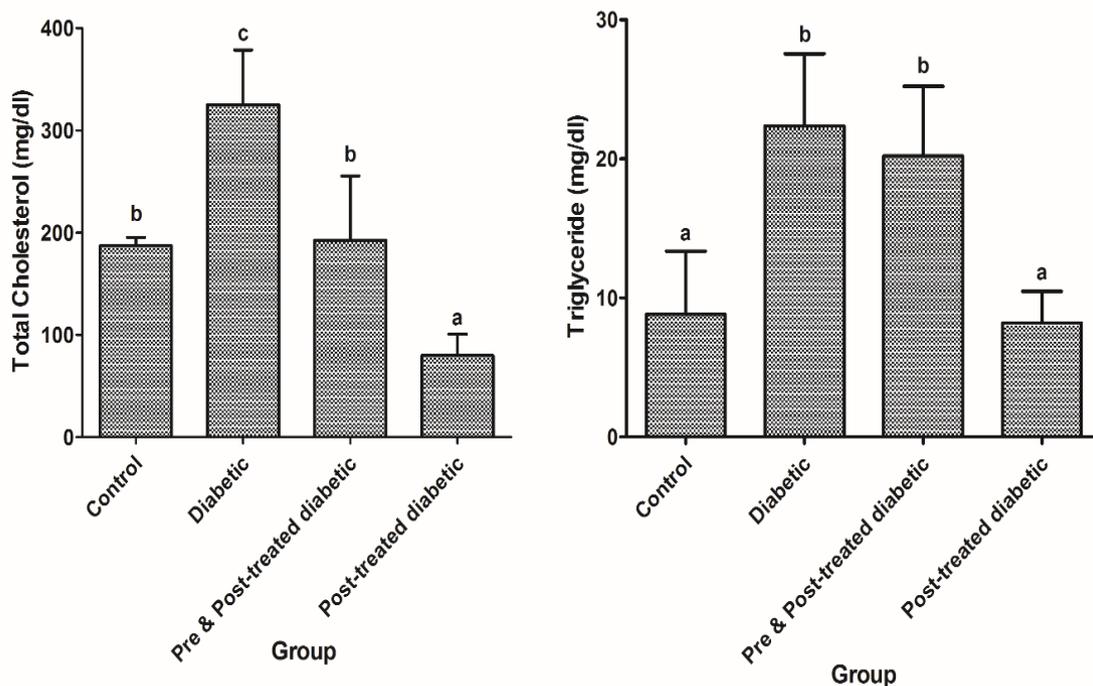


Fig. 1. Effect of aqueous extract of *Syzygium aromaticum* on lipid profile

Values are mean weight ± SEM (n=5). Different letters along the column and across the row indicate significant difference at $p < 0.05$

Syzygium aromaticum treatment significantly reduced blood glucose level in post treated diabetic rats on the 7th and 14th day of administration which represents reversal of insulin resistance or increasing insulin secretion possibly by regeneration of damaged pancreatic β -cells in alloxan-induced diabetic rats. These effects may be attributed to either inhibition of increased insulin output, inhibition of the intestinal absorption of glucose and increase metabolism because *S. aromaticum* contains alkaloids, tannins and glycosides which have been proved to be antidiabetic activity by different mechanisms of action [25]. Hyperlipidemia is one of the major cardiovascular

risk factors. Hyperlipidemia, a recognized complication of diabetes mellitus characterized by elevated level of diabetic control which may be due to its protective effect in controlling wasting i.e., reversal of gluconeogenesis and may also be due to the improvement in insulin secretion and glycemic control. The extract has been proven in literature to reduce blood cholesterol and triglyceride level. The significant reduction in cholesterol and triglyceride levels in post treated diabetic group when compared with the diabetic control rats supports the findings of Coon and Emst [26] who stated that most hypoglycemic plants have potentials of ameliorating diabetic lipid metabolism anomalies.

Table 2. Effect of Aqueous extract of *Syzygium aromaticum* on blood glucoses (mg/dl)

Groups	Initial day	3 rd day	7 th day	14 th day
Control	99.0±9.42 ^{a,x}	108.0±5.38 ^{a,x}	75.2±7.30 ^{a,x}	64.0±4.01 ^{a,x}
Diabetic control	96.8±5.89 ^{a,x}	230.4±41.94 ^{b,y}	181.6±43.9 ^{b,y}	105±43.82 ^{b,x}
Pre-treated & Post-treated diabetic group	106.8±9.50 ^{a,x}	232.8±73.78 ^{b,y}	89.6±13.28 ^{a,y}	72.0±4.06 ^{a,x}
Post treated diabetic group	103.8±4.53 ^{a,x}	296.8±72.84 ^{b,y}	157.0±53.74 ^{a,y}	102.2±12.11 ^{a,y}

Values are mean weight \pm SEM (n=5). Different letters along the column and across the row indicate significant difference at $p < 0.05$

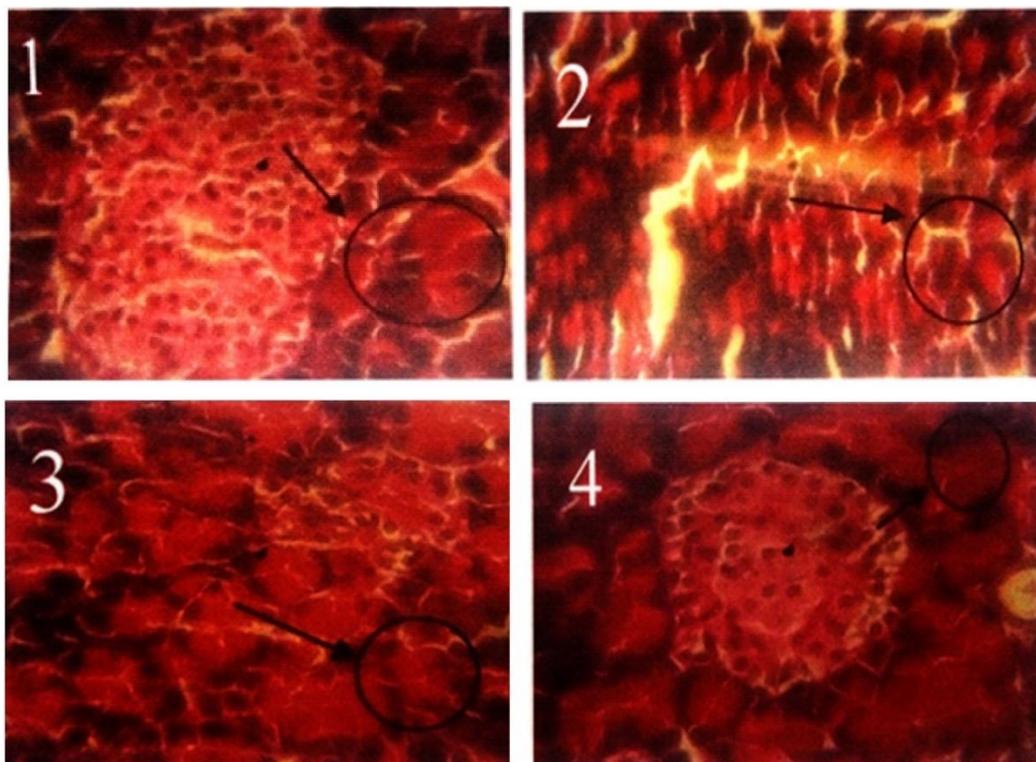


Fig. 2. Photomicrograph of the pancreas of Wistar rats at X400 magnification (1) Control group with no degenerative changes, (2) Diabetic control with degeneration of β -cells, (3) Pre and post treated group with regeneration of β -cells, and (4) Post treated group with regeneration of β -cells

The result of the present study investigation of *Syzygium aromaticum* showed significant antidiabetic activity properties against alloxan-induced diabetic rats.

5. CONCLUSION

Findings of the present study suggest that aqueous *S. aromaticum* seeds extract at dose of 500 mg/kg body weight brings about significant beneficial effects in various physiological/histopathological parameters against alloxan-induced diabetes.

CONSENT

Not applicable.

ETHICAL APPROVAL

This study was carried out according to the guidelines of National Research Council Guide and in accordance with the principles of Good Laboratory procedure (GLP) following approval of the Institutional Ethical Committee on the Use and Care of Animals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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