IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 8, Issue 8 Ver. II (Aug. 2014), PP 36-40
www.iosrjournals.org

Anti-Diabetic Effect of a Herbal Tea Processed from 'Nchanwu' (Ocimum gratissimum) Leaves on Alloxan-Induced Diabetic Rats

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Abstract: *Anti-diabetic effect of a herbal tea processed from Ocimum gratissimum was evaluated on five groups of rats; N, D, C1, C2 and C3. Group N and D were non-diabetic and diabetic rat groups fed with rat feed and tap water while groups C1, C2, and C3 were diabetic rats fed with rat feed and different concentrations of the herbal tea; 2g/200ml, 4g/200ml and 6g/200ml respectively. The feed, water and the herbal tea were provided adlibitum for the respective groups for a period of 21 days. The herbal tea contained 17.29% moisture, 1.70% crude fat, 28.9% crude protein, 11.1% fibre, 5.9% ash and 35.93% carbohydrate. At the end of the treatment period, groups C1, C2 and C3 had 50.2%, 70.1% and 25.4% reduction while groups N and D had 1% and 18.8% increment in their fasting blood glucose concentrations respectively. The histological result showed that all the groups that took different concentrations of the herbal tea had larger sizes of islet cells when compared with that of the diabetic group D; suggesting that the herbal tea at these different concentrations may have induced proliferation of the remnant of the islet cells that escaped the damage caused by the alloxan injection.*

Keywords: *alloxan, anti-diabetic, herbal tea, histology, Ocimum gratissimum*

I. Introduction

Medicinal plants are plants that contain inherent active ingredients used to cure diseases or relieve pain [1]. According to World Health Organization (WHO), the majority of the world's human population especially in developing countries, depend on traditional medicine for primary health care [2]. Medicinal plants have demonstrated their contributions to the treatment of diseases such as HIV/AIDS, malaria, diabetes and sickle-cell anaemia, mental disorders, microbial infections and many others. [3-5].

Diabetes Mellitus which is a non-communicable disease, considered as one of the five leading causes of death in the world, is a disease associated with glucose metabolism resulting from defects in insulin secretion and action [6,7]. About 100 million people around the world have been diagonized with diabetes and by the year 2010, it was projected that 215 million people will have the disease [8]. There is yet no effective cure for diabetes, rather a lifelong treatment and management, which the patients have to put up with, and the available drugs and insulin currently in use, in the management of this disease are all associated with several undesirable side effects [9-11]. The undesirable side effects and high cost of anti-diabetic drugs have led to search for plants with hypoglycemic properties and their employment in the management of diabetes [12].

Ocimum gratissimum is a minty aromatic plant grown in many parts of Nigeria as a condiment or spice used in many food preparations. It is known as 'nchanwu'(Igbo), 'efiri' (Yoruba) and 'dai-doya ta gida' (Hausa). Traditionally, it is used as a medicinal plant in the treatment of headache, diarrhea, wart, worms and kidney function [13,14]. Literature has also shown that aqueous extract of this plant possesses anti-hyperglycemic effect [15-17]. Transforming this wonderful plant into a herbal tea where it will serve as a food as well as an anti-diabetic therapy will be a great relief to the diabetic patient, for the life-long treatment and management of diabetes mellitus.

The aim of this work therefore, is to process the leaves of Ocimum gratissimum plant into a herbal tea and to evaluate the anti-diabetic effect of this herbal tea on alloxan-induced diabetic rats.

II. Materials and Methods

2.1 Materials

Fresh Ocimum gratissimum leaves were purchased from Owerri Main market, Imo state, Nigeria and was authenticated at Department of Crop Science, Federal University of Technology, Owerri, Nigeria.

2.2 Processing of Ocimum gratissimum Herbal Tea

The herbal tea was produced using a modified method of processing of green tea as described by [18]. Ocimum gratissimum leaves were picked, washed, drained, pan fried, coarsely ground and oven dried at 40^{o}C. The herbal tea produced was packaged for subsequent usage and analysis.

2.3 Proximate Analysis

The herbal tea sample was analyzed for proximate composition (moisture, fat, crude protein, crude fibre, ash) using the methods described by AOAC [19]. Carbohydrate content was determined by difference.

2.4 Animals

Total of 50 male albino rats (Rattus norvegicus) of wister strain were sourced from a veterinary clinic Owerri, Imo state. Upon arrival, the animals were acclimatized for 7 days while been maintained on regular commercial rat feed. Tap water and feed were provided adlibitum throughout the period

2.5 Induction of Experimental Diabetes

After one week of acclimatization, the rats were subjected to a 12-hour fast after which diabetes [type 1 diabetes mellitus] was induced in 45 rats with alloxan monohydrate dissolved in Saline (0.9% W/V of NaCl) given at a dose of 120mg/kg intraperitoneally. However, the remaining 5 rats which served as non-diabetic rats received Saline injection also given intraperitoneally. 3 days after injection of alloxan monohydrate, diabetes was confirmed in alloxan-treated rats with fasting blood glucose concentration above 250mg/dl [20].

2.6 Experimental Design

On treatment day zero (3 days after induction of diabetes) initial blood glucose concentration was measured in non-diabetic (normal) and alloxan-induced diabetic rats. The diabetic rats were divided into 4 groups as follows: Group D, C1, C2 and C3, while Group N served as the non-diabetic rats. Groups N and D which were non-diabetic and diabetic rats received only tap water and commercial rat feed. Groups C1, C2 and C3 which were diabetic groups received different concentrations of the herbal tea: 2g/200ml, 4g/200ml and 6g/200ml respectively along with commercial rat feed. The treatment lasted for 21 days from the day of confirmation of diabetes.

2.7 Blood Glucose Determination

Blood samples were collected from the tail veins of the non-diabetic (normal) and alloxan-induced diabetic rat groups on treatment day zero (3 days after induction of diabetes) and at the end of treatment period (21 days from start of treatment) as indicated in the experimental procedure. Blood glucose was determined in these blood samples using a Glucometer (One Touch Ultra Blood Glucose Monitoring System).

2.8 Histological Examination

At the end of treatment period (21 days from start of treatment) the animals were slaughtered and their peritoneum stripped open to harvest the pancreas. The tissues were quickly processed histologically and haematoxylin using eosin staining technique [21].

III. Results

3.1 Proximate composition

The result of the proximate composition of the Ocimum gratissimum tea is shown in Table 1. The percentage moisture content of the herbal tea (17.29%) was close to the value (12.0%) obtained by Asaolu et al.,[22] for sundried Ocimum gratissimum leaves. The fibre content of the herbal tea (11.1%) was also in agreement with the findings of Isong and Idiong [23]. However the crude protein and crude fat contents of the herbal tea; 28.09% and 1.7% respectively were lower than the values reported by Asaolu et al.,[22] for sundried Ocimum gratissimum leaves.

Table 1. Proximate Composition of Ocimum gratissimum tea

Moisture (%)	Crude Fat (%)	Crude Protein (%)	Fibre (%)	Ash (%)	Carbohydrate (%)
17.29±0.59	1.70±0.17	28.09±0.16	11.10±0.26	5.90±0.10	35.93±0.86

3.2 Blood Glucose Concentration

The initial and the final mean fasting blood glucose concentrations of the different treatment groups are shown in Fig 1. The initial mean fasting blood glucose concentrations of the different treatment groups on treatment day zero (day of confirmation of diabetes) were compared and the results showed that the mean fasting blood glucose concentrations of the alloxan-induced diabetic rats (D, C1, C2, C3) were more than 5 times higher than those of the non-diabetic rats (N). The diabetic rats that drank different concentrations of the herbal tea (C1, C2 C3) showed a remarkable decrease in their mean fasting blood glucose concentrations when compared with their initial mean values or with that of the diabetic group D. There was no remarkable change in the initial and final mean fasting blood glucose concentration of the non-diabetic group N. However, the

diabetic group D which did not take the herbal tea showed a remarkable increase in its final mean fasting blood glucose concentration when compared with the initial value.

The percentage reduction in blood glucose of the different treatment groups is shown in Table 2. Diabetic groups that received 2g/200ml, 4g/200ml and 6g/200ml of the herbal tea, had percentage reduction of 50.2%, 70.1% and 25.4% respectively while the non-diabetic and diabetic groups that did not receive the tea, had percentage increment in blood glucose of 1% and 18.8% respectively.

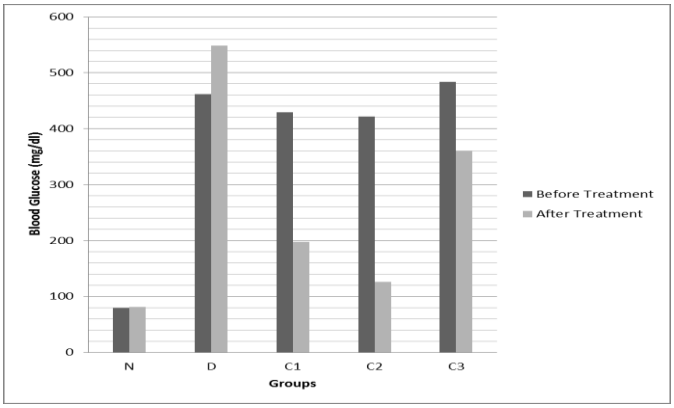

Figure 1: Blood glucose levels before and after treatment for different treatment groups.

Table 2. Percentage Increment/Reduction of Blood Glucose of the Different Treatment Groups

Group	Treatment	Percentage Reduction (%)
N	Non Diabetic Control	+ 01.0
D	Diabetic Control	+ 18.8
C1	Diabetic/ Conc I	- 50.2
C2	Diabetic/ Conc II	- 70.1
C3	Diabetic/ Conc III	- 25.4

N - Non-diabetic control group fed with rat feed and tap water
D - Diabetic group fed with rat feed and tap water
C1 - Diabetic group fed with rat feed and 2g/200ml of herbal tea
C2 - Diabetic group fed with rat feed and 4g/200ml of herbal tea
C3 - Diabetic group fed with rat feed and 6g/200ml of herbal tea

3.3 Histological Results

Plate 1 shows the pancreatic cells of the non-diabetic group N. This group received commercial rat feed and tap water only during the 21days of treatment. In this group the islet of langerhan and their cells (α, β and ϒ cells) were well preserved. The serous acini cells were also demonstrated and well preserved. The pancreatic cells of the diabetic group D are shown in Plate 2. This group of rats was diabetic and received commercial rat feed and tap water only during the treatment period. The pancreatic specimen showed a reduction in the size of the islet and the surrounding serous acini cells; with infiltration of the cells of the islet into the acini environment. In Plate 3, the cells of the pancreatic specimen for diabetic group C1 were shown. Group C1 was diabetic and received commercial rat feed and 2g/200ml of herbal tea during the treatment period. The specimen showed an islet similar to the non-diabetic group N. The acini and the intra lobular duct were also outlined. Some cells of the islet were scattered around the acini cells. Plate 4 shows the pancreatic cells of diabetic group C2. This group of rats was diabetic and received commercial rat feed and 4g/200ml of herbal tea during the treatment period. The islets in this specimen were similar to those of the diabetic group C1 (Plate 3). The serous acini cells were well outlined similar to those of the non-diabetic group N. Also there was infiltration of cells of islet into the tissue. The pancreatic cells of rats in diabetic group C3 are shown in Plate 5. This group of rats was also diabetic and received commercial rat feed and 6g/200ml of herbal tea during the treatment period. The specimen showed similar histological features as observed in diabetic group C2 (Plate 4)

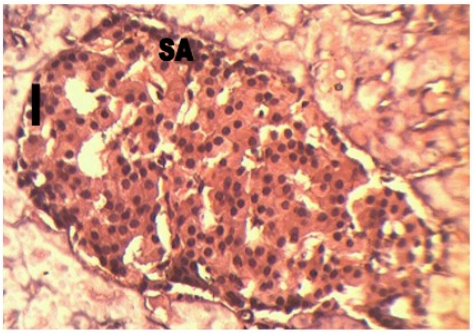

PLATE 1.Group N (Normal control) received water only
I – Islet of langerhans, SA – Serous acinii cells.

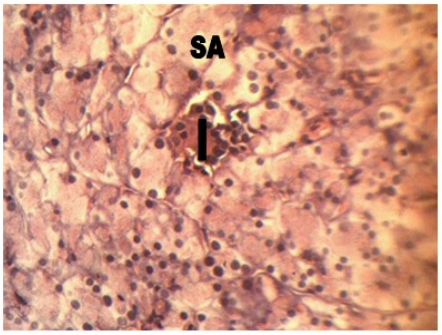

PLATE 2.Group D (Diabetic control) received water only
I – Islet of langerhans, SA – Serous acinii cells.

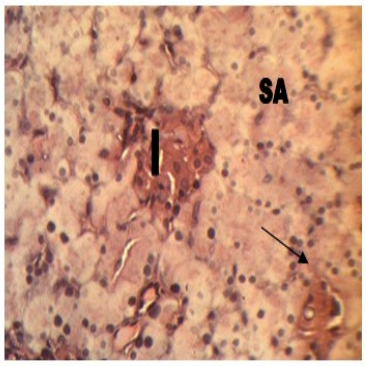

PLATE 3. Group C1 (Diabetic) received 2g/200ml of herbal tea
I – Islet of langerhans, SA – Serous acinii cells.

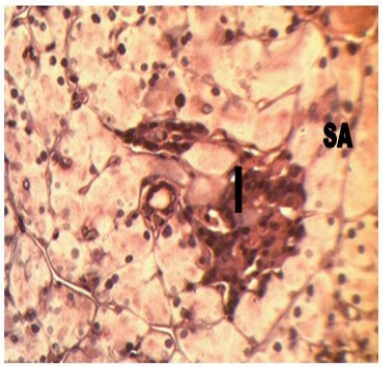

PLATE 4.Group C2 (Diabetic) received 4g/200ml of herbal tea
I – Islet of langerhans, SA – Serous acinii cells.

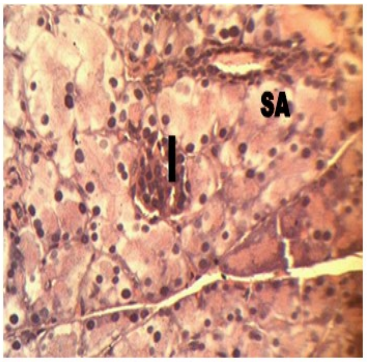

PLATE 5.Group C3 (Diabetic) received 6g/200ml of herbal tea
I – Islet of langerhans, SA – Serous acinii cells.

IV. Discussion

Diabetes mellitus is characterized by reduced capacity of β-cells of the pancreas to secrete sufficient insulin to induce the activity of glucose metabolizing enzymes, (hexokinase, phosphofructokinase, glucose-6-phosphate dehydrogenase, glucokinase) whether the cells are destroyed as in type 1 diabetes (IDDM) or intact as in type 2 diabetes (NIDDM) [24]. Reduced activity of these glucose metabolizing enzymes have been associated with the condition of diabetes mellitus [25-28]. However, some plant extracts have been shown to increase the activity of these enzymes, thus increasing the flux of glucose into the glycolytic pathway and pentose monophosphate shunt leading to decrease in blood glucose [15, 17, 29, 30].

In this study, the anti-diabetic property of Ocimum gratissimum herbal tea was evaluated. The result showed that all the diabetic groups that received different concentrations of the herbal tea had significant reduction in their fasting blood glucose concentrations when compared with their initial fasting blood glucose values or with the diabetic group D that did not take the herbal tea. This result is similar to the result obtained by Mohammed et al. [17] who injected extracts of Ocimum gratissimum plant on diabetic rats and obtained 81.03% reduction in fasting blood glucose. In this work, a 70.1% reduction in fasting blood glucose concentration was obtained with 4g/200ml of Ocimum gratissimum taken as herbal tea, by alloxan-induced diabetic rats. The histological examination also showed (Plates 1-5) that all the groups that received different concentrations of the herbal tea (C1, C2, C3) had larger size of islet cells when compared with the size of islet cells of the diabetic group D that did not take the herbal tea, suggesting that the herbal tea at these different concentrations may have induced the proliferation of the remnant of the islet cells which were not damaged by the alloxan. The group that took 4g/200ml of the herbal tea showed the largest size of islet cell when compared with the other diabetic groups that took 2g/200ml and 6g/200ml of the herbal tea, showing that better effect was achieved at this concentration than at other concentrations.

V. Conclusion

Ocimum gratissimum leaves have been shown to possess anti-diabetic property. Transforming the leaves of this plant into a herbal tea, which serves as a food as well as an anti-diabetic therapy will be a welcomed development for a diabetic patient who lives with this disease, from the time it surfaces to the remaining part of his life.

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