EFFECT OF AQUEOUS LEAF EXTRACT OF *OCIMUM GRATISSIMUM* IN ALLOXAN-INDUCED DIABETIC WISTAR RATS

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ABSTRACT

The effect of aqueous extract of leaves of *Ocimum gratissimum* in alloxan- induced diabetes Wister rats was investigated. Sixteen (16) adult male albino Wistar rats were randomly divided into four groups (A-D). Group A and C were administered alloxan (150mg/kg) intraperitionally to induce diabetes in them. Groups A and Group B were later treated with the aqueous extract (400mg/kg) for 22 days, while groups C and D served as negative and positive controls respectively. The extract treated diabetic rats group showed significant improvement in body weight and the blood glucose levels decreased significantly ($P< 0.05$) when compared with the diabetic control group. Blood cholesterol and triglycerides level decreased significantly ($P< 0.05$) in diabetic rats group treated, except for the HDL-cholesterol level which increased when compared with the diabetic control group. The extract treated groups showed no significant difference ($P>0.05$) in AST and ALT activity when compared with the normal control group except for ALP activity which decreased when compared with the normal control. Also, PCV and RBC level increased significantly ($P< 0.05$) in diabetic rats group treated as compared with the diabetic control group untreated. This study showed that the leaf extract posses hypoglycaemic and hypocholesterolemic activity as claim by Nigerian traditional herbal medicine practitioners that *Ocimum gratissimum* leaves have hypoglycaemic properties.

keywords: *Ocimum gratissimum,* Diabetes mellitus, Alloxan, hypoglycaemia

INTRODUCTION

Diabetes mellitus is a condition in which a person has a high blood glucose level, either because the body doesn't produce enough insulin, or because body cells don't properly respond to the insulin that is produced [1]. It is a syndrome of chronic hyperglycaemia due to relative insulin deficiency, resistance, or both. It affects more than 120 million people world-wide and it is estimated that it will affect 220 million people by the year 2020 . Diabetes is characterized by hyperglycemia together with biochemical alteration of glucose and lipid metabolism. These traits are hypothesized to be responsible for the damage to cell membrane, which in turn result in an elevated production of Reactive Oxygen Species (ROS) and the simultaneous decline in antioxidants defense mechanism observed in diabetic patients could promote the development of later complications [2].

These late complications of diabetes results in reduced life expectancy and major health costs. These include macrovascular disease, leading to an increased prevalence of coronary artery disease, peripheral vascular disease and stroke as well as microvascular damage causing diabetes retinopathy, nephropathy and neuropathy which is another major complication.

However, in modern medicine, satisfactory effective therapy is not yet available to cure diabetes mellitus [3]. Unfortunately, neither insulin therapy nor oral anti-diabetic drugs (like sulphonylureas, biguonidies, metformin, acarbose and thiazolinediones) reinstate a normal pattern of glycaemia control; whether used alone or in combinations, or administered as a standard or intensive regime [4] Group, 1995). Oral hypoglycemic drugs play important role in the treatment of non-insulin dependent diabetes mellitus (NIDDM) and have characteristic profile of side effects [5]. The wide gap for additional agents to combat hyperglycemia and its accompanying.

MATERIALS AND METHODS

Experimental Animals

Sixteen (16) adult male white albino Wistar rats (150g-210g) were obtained from the Animal House Unit of the Department of Pharmacology, University of Jos. The rats were fed with standard feed (vital growers mesh) from Grand Cereal and Oil mills limited, Jos and water ad libitum. The feed nutrient composition includes; crude protein 14.5%, fat 7.0%, crude fiber 7.4%, calcium 0.8% and phosphorous 0.4%. The rats were allowed 3 days to accumulate before the start of the experiments.

Collection, Identification and preparation of Plant Materials

Fresh leaves of *Ocimum gratissimum* were collected from Liberty Dam Road, Jos. The leaves were identified and authenticated as *Ocimum gratissimum* leaves at Federal College of Forestry, Jos. Fresh leaves of *Ocimum gratissimum* were air dried and blended into coarse powder. The powder was poured into two conical flasks, mixed properly with water and shake for about 2 hours with a mechanical shaker. After shaking, the mixture was warmed for few minutes and then allowed to cool. The mixture was then filtered through a fine sieve; the filtration was done several times until, complete fine filtrate was obtained. The filtrate was poured into a beaker and placed on a water bath to evaporate to dryness.

Induction of Diabetes in Experimental Animals

Group A and C rats, were induced with diabetes by intraperitoneal injection of alloxan solution at a dose of 150mg/kg body weight.

Treatment with *Ocimum gratissimum* Extract Solution

Two days after diabetes was induced in group A and C rats, group A and B rats were treated. The treatment was done through oral administration of *Ocimum gratissimum* leaves extract solution, at a dose of 400mg/kg body weight daily. This treatment last for 22 days.

Experimental Design

Sixteen (16) healthy adult male albino rats weighing between 150-210g were used in this study. They were randomly divided into four groups (A-D) with four rats in each group. The grouping is as follows:

Group A: Diabetic rats, treated with *Ocimum gratissimum* leaves extract solution and fed for 22 days.

Group B: Normal rats treated with *Ocimum gratissimum* leaves extract solution and fed for 22 days.
Group C: Diabetic control rats' untreated (Negative control) and fed for 22 days.
Group D: Normal control rats' untreated (Positive control).

Collection and Preparation of Blood Sample

At the end of the 22 days of treatment with *Ocimum gratissimum* leaf extract solution, blood samples were collected from the tail vein of the rats (both treated and untreated groups), for haematological analysis. After which blood was finally collected from the jugular vein into centrifuge tubes. The blood samples in each centrifuge tubes were allowed to clot and serum separated by centrifugation at 5,000 rpm for 10 minutes. The serum was used for biochemical analysis.

Analysis of Biochemical Parameters

Determination of Blood Glucose Level by Enzyme Method

Blood glucose level was determined using kit product of fortress diagnostics [6].

Determination of Serum Total Cholesterol level by Enzyme Method.

Serum total cholesterol level was determined using kit product of randox diagnostics [7]

Determination of Serum High Density Lipoprotein (HDL- Cholesterol) level

Serum HDL – cholesterol was determined using kit product of fortress diagnostics [8].

Determination of Serum Triglycerides Level

Serum triglycerides level was determined using kit product of stambio diagnostics [9].

Determination of Serum Alanine Aminotransferases (ALT) Activity

Serum ALT activity was determined using fortress diagnostics kit product [10].

Determination of Serum Aspartate Aminotransferases (AST) Activity

Serum AST activity was determined using fortress diagnostics kit production [10].

Determination of Serum Alkaline Phosphatase (ALP) Activity

Serum ALP activity was determined using kit product of quimica clinica aplicada [11].

Analysis of Haematological parameters

Determination of Packed Cell Volume (PCV)

The packed cell volume (PCV) was determined using micro-haematocrit method.

Determination of Red Blood Cell Count (RBC)

0.995 ml of Hayem's solution was dispensed into test tubes, followed by the addition of 0.005ml of blood. The cover slip was placed over the counting chamber and the diluted blood was mixed and introduced into the counting chamber at an angle of 45^{o}.The chamber was focus on microscope for counting under X40 objective lens, to count the red blood cells.

Statistical Analysis

The results were expressed as mean ± standard error of mean (s.e.m) for four rats in each group and all grouped data were statistically analyzed using Students'-test. $P<0.05$ was considered significant while $P>0.05$ was considered insignificant.

RESULTS

Weight Changes of Rats

Table 1 shows the body weight of the rats on day 1 and 22 (final) day of the experiment, as well as the percentage (%) weight gain/loss of the rats during the period of the experiment. Group A, B and D rats showed significant weight gained while group C rats showed significant weight loss.

Table 1: Weight changes of Rats in Grams (g)

Group	Initial Weight	Final Weight	(%) Weight Gain/Loss
Diabetic + Extract	163.75 ± 12.50	198.25 ± 1.90	21.07
Normal + Extract	156.50 ± 4.43	197.50 ± 1.12	26.20
Diabetic Control	159.25 ± 7.89	142.33 ± 1.50	-10.62
Normal Control	150.33 ± 6.43	173.67 ± 7.09	15.53

Values are expressed as mean ± s.e.m for, n=4

Blood Glucose Level

Table 2 shows the blood glucose level (mmol/L) in the diabetic and normal rats groups. Induction of diabetes in the experimental rats was confirmed by the significant increased ($P< 0.05$) in blood glucose level of the diabetic control rats group when compared with the normal control rats group. Following the oral treatment with *Ocimum gratissimum* leaf extract, the blood glucose level of the diabetic rats group treated decreased significantly ($P< 0.05$) when compared with the diabetic control rats group.

Table 2: Effect of aqueous extract of *Ocimum gratissimum* on Serum Glucose Level in Normal and Diabetic Rats.

Groups	Blood glucose level (mmol/L)
Diabetic + Extract	5.05 ± 0.94 ^{a,b}
Normal + Extract	4.48 ± 0.80 ^{b}
Diabetic Control	11.95 ± 1.21 ^{c}
Normal Control	4.98 ± 0.18

Valies are expressed as mean ± s.e.m for 3 determinations, n=4.
a = Statistically significant decrease (P< 0.05) when compared with diabetic control.
b = Statistically insignificant difference (P> 0.05) when compared with normal control.
c = Statistically significant increase (P< 0.05) when compared with normal control.

Serum Lipid Profile

Table 3 shows the blood lipid profile (mmol/L) in the diabetic and normal rats groups. In the diabetic control rats groups, the cholesterol and triglycerides levels increased significantly (P< 0.05) when compared with the normal control rats group. In diabetic rat group treated with *Ocimum gratissimum* leaf extract, the blood cholesterol and triglycerides levels decreased significantly (P< 0.05) when compared with the diabetic control group untreated, except for high density lipoprotein (HDL) – cholesterol which increased. The normal rats group treated with the extract shows no significant difference (P> 0.05) in blood lipid profile when compared with the normal control group.

Table 3: Effect of aqueous extract of *Ocimum gratissimum* on Serum Lipid Profile in Normal and Diabetic Rats.

Groups	Cholesterol (mmol/L)	HDL (mmol/L)	Triglycerides (mmol/L)
Diabetic + Extract	4.70 ± 0.12 [a,c]	4.81 ± 0.22 [a,b]	0.82 ± 0.10 [a,b]
Normal + Extract	3.43 ± 0.82 [c]	6.65 ± 1.17 [b]	0.49 ± 0.24 [b]
Diabetic Control	8.93 ± 1.24 [d]	1.38 ± 0.28 [d]	1.62 ± 0.46 [d]
Normal Control	5.02 ± 0.20	5.13 ± 1.41	0.61 ± 0.15

Values are expressed as mean ± s.e.m for 3 determinations, n=4
a = Statistically significant decrease (P< 0.05) when compared with diabetic control except HDL – Cholesterol which increased.
b = Statistically insignificant difference (P> 0.05) when compared with normal control.
c = Statistically significant decrease (P< 0.05) when compared with normal control.
d = Statistically significant increase (P< 0.05) when compared with normal control except HDL – Cholesterol which decreased.

Liver Enzymes Activity

Table 4 shows liver enzymes activities of the normal and diabetic rats groups. In diabetic control rats group, AST and ALT activity increased significantly (P< 0.05) when compared with the normal control except for ALP activities which shows no significant different (P> 0.05). The diabetic rats group and the normal rats group treated with the extracts show no significant difference in AST and ALT activity when compared with the normal control group untreated, except for ALP activities which decreased significantly (P< 0.05) when compared with the normal control group.

Table 4: Effect of aqueous extract of *Ocimum gratissimum* on Liver Enzymes Activity in Normal and Diabetic Rats.

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Diabetic + Extract	12.68 ± 1.12 a,b	6.55 ± 1.13 a,b	26.01 ± 1.64 a,b
Normal + Extract	11.90 ± 0.49 b	6.55 ± 1.10 b	25.56 ± 0.91 b
Diabetic Control	15.78 ± 0.67 c	8.73 ± 1.19 c	31.72 ± 1.34 c
Normal Control	12.70 ± 1.03	5.36 ± 0.34	31.06 ± 1.31

Values are expressed as mean ± s.e.m for 3 determinations, n=4

a = Statistically significant decrease (P< 0.05) when compared with diabetic control except.
b = Statistically insignificant difference (P> 0.05) when compared with normal control except ALP which decreased.
c = Statistically significant increase (P< 0.05) when compared with normal control except ALP which shows no significant difference.

Haematological Parameters

Table 5 below shows the haematological parameters; packed cell volume (PCV) and Red blood cell count (RBC) of the normal and diabetic rats group. In diabetic control rats group, PCV and RBC decreased significantly when compared with the normal control rat group. The diabetic rat group treated with the extract show insignificant difference (P> 0.05) in the PCV and RBC level when compared with the normal control rats group.

Table 5: Effect of aqueous extract of *Ocimum gratissimum* on PCV and RBC RBC

Groups	PCV (%)	RBC (mm^3)
Diabetic + Extract	55.00 ± 1.73 a,b	6032.50 ± 86.32 x 10^3 a,b
Normal + Extract	59.30 ± 2.31 b	6540.00 ± 42.10 x 10^3 c
Diabetic Control	49.00 ± 4.36 d	5266.70 ± 28.15 x 10^3 d
Normal Control	55.36 ± 3.06	6113.30 ± 58.82 x 10^3

Values are expressed as mean ± s.e.m for 3 determinations, n=4 rats
a = Statistically significant increase (P< 0.05) when compared with diabetic control.
b = Statistically insignificant difference (P> 0.05) when compared with normal control.
c = Statistically significant increase (P< 0.05) when compared with normal control.
d = Statistically significant decrease (P< 0.05) when compared with normal control.

Discussion

Diabetic control rats group showed significant weight loss as indicated by the percentage weight loss (Table 1). The observed weight loss was consistent with the low level of feed intake as the rats showed poor appetite during the experiment. Besides, the weight loss may be due to fluid depletion and accelerated breakdown of fats and adipose muscle. Diabetic and normal rats group (Group A and B) treated with the extracts showed significant percentage weight gain than the normal control group which was untreated (Table 1). These may be due to the presence of several phytochemicals such as alkaloids and flavonoids in the extract, this phytochemicals are believed to have facilitated glucose utilization by peripheral tissues.[12].

Alloxan monohydrate, a pyrimidine derivative is a very selective toxin of pancreatic β-cells, through its inhibition of glucokinase, thus making it a good model for screening plant with antidiabetic properties[13]. Induction of diabetes with alloxan was confirmed by the significant increased ($P< 0.05^{c}$) in blood glucose level of the diabetic control group when compared with the normal control (Table 2). Treatment with the aqueous extract of O*cimum gratissimum* significantly decreased ($P<0.05^{a}$) the blood glucose level of the diabetic rats group treated when compared with diabetic control. The normal rats group treated with the extract shows no significant difference ($P> 0.05^{b}$) in blood glucose level when compared with normal control group. This finding is in line with the result of [12].

Blood cholesterol and triglycerides level increased significantly ($P< 0.05$) in diabetic control group except for HDL – Cholesterol level which decreased when compared with normal control group (Table 3). The increase in blood cholesterol and triglycerides is due to the action of hormone sensitive lipase, which promotes lipolysis and subsequently increases the level of plasma free fatty acids and triglycerides. These free fatty-acids are catabolysed to acetyl CoA which is further channeled to cholesterol synthesis thus, increasing blood cholesterol level.

Treatment with the aqueous extract, significantly decreased ($P< 0.05$) blood cholesterol and triglyceride levels in the diabetic rats group treated, except for HDL cholesterol level which increased close to normal. And in normal rats group treated with the extract, blood cholesterol level decreased significantly ($P< 0.05$) when compared with normal control.

The aqueous extract shows no significant toxic effect on vital body organs like the liver as determined by the liver enzymes biochemical indices (Table 4). Diabetic control group showed significant increase ($P< 0.05$) in AST and ALT activity when compared with normal control group except for ALP activity which was insignificantly different when compared with normal control group.

Treatment with the leave extract show no significant difference ($P> 0.05$) in AST and ALT activity in both diabetic and normal rats groups treated, except for ALP activity which significantly decreased when compared with normal control group.

PCV and RBC level decreased significantly (P< 0.05) in diabetic control group when compared with normal control group (Table 5).However, treatment with the aqueous extract significantly increased the PCV and RBC levels in diabetic rats group treated, while the normal rats group which was also treated with the extract, shows no significant difference in PCV level except RBC level which increased significantly
(P< 0.05) when compared with the normal control group.
At the end of this study, the investigation results shows that aqueous extract of the leaf of *Ocimum gratissimum* posses hypoglycaemic and hypolipidemia properties.

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