

Full Length Research Paper

Anti-diabetic action of the aqueous extract of *Ocimum suave* in alloxan-induced diabetic rats

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The anti-diabetic action of the aqueous extract of *Ocimum suave* in alloxan-induced diabetic rats was investigated. Male Wister strain albino rats (120 to 150 g) were divided into five groups of five animals each, three of which were made diabetic using alloxan. All rats in the diabetic groups had initial fasting blood glucose (FBG) levels ≥ 150 mg/dl, but after three weeks of treatment with extract or glibenclamide; the FBG significantly ($p < 0.05$) dropped between 44 and 58%, as compared to the 13% drop recorded in the untreated diabetic rats. Diabetes also caused a significant ($p < 0.05$) decrease in packed cell volume which was completely prevented by administration of the extract. Untreated diabetes caused significant increases in serum total - and high density lipoproteins (HDL)-cholesterol (CHL), while significantly reducing the serum triacylglycerols (TG). Treatment with *O. suave* extract caused significant increase in the serum levels of TG and HDL-CHL, above the diabetic levels; while it reduced ($p < 0.05$) total CHL to values lower than normal. Liver thiobarbituric acid reactive substances (TBARS) was unaffected by diabetes but it was significantly ($p < 0.05$) increased in the kidney; this increase was completely prevented by treatment with the extract. The superoxide dismutase (SOD) activity in the kidney was significantly reduced by diabetes but it was further reduced by administration of the extract to diabetic rats. The serum levels of urea and alanine transaminase were significantly increased above normal in untreated diabetic rats; treatment with the extract did not significantly change the situation. It is concluded that the aqueous extract of *O. suave* has anti-hyperglycemic and hypolipidemic effects as well as antioxidant action in alloxan-induced diabetic rats.

Key words: *Ocimum suave*, diabetes mellitus, anti-hyperglycemic, oxidative stress, anti-oxidant.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of the endocrine system. The disease is characterized by hyperglycaemia caused by either insulin insufficiency or insensitivity of target cells to insulin; both leading to impaired metabolism of glucose, lipids and proteins as well as dysfunction and failure of various organs (Kim et al., 2006). The disease occurs worldwide and its incidence is increasing rapidly in most parts of the world

(Adebayo, 2009). Diabetes is commonly accompanied by other cardiovascular risk factors such as dyslipidemia, hypertension, prothrombic factors and microvascular problems involving eyes, kidney and peripheral nerves (Barnett and O' Gara, 2003). Experimental evidence is mounting strongly associating oxidative stress and most of the complications seen in DM (Seth and Sharma, 2004; Devasagayam et al., 2007). The oral hypoglycaemic agents currently used in clinical practice have characteristic profiles of serious side effects such as weight gain, gastrointestinal discomfort and nausea, liver failure and diarrhea (Suba et al., 2004; Stephen, 2006); in addition to being rather costly. However, DM is also treated traditionally in some places using anti-diabetic medicinal plants (Kim et al., 2011). *Ocimum* species are culinary herb plants that belong to the family

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Abbreviations: FBG, Fasting blood glucose; HDL, high density lipoproteins; CHL, cholesterol; TG, triacylglycerols; TBARS, thiobarbituric acid reactive substances; SOD, superoxide dismutase.

Lamiaceae. The local Nigerian names are: "Dan doya ta gida" (Hausa), "Efirin" (Yoruba) and "Ncha-anwu" (Ibo). They are important ingredients in many folk remedies for stomach ache, cough, convulsions, diarrhea, gout, toothache and gastric ulcers (Seung-Jo-Lee et al., 2004) as well as diabetes and associated cardiovascular complications (Zeggwagh et al., 2007).

The essential oils of these plants have been shown to have antimicrobial, anticancer and antioxidant properties (Simon et al., 1990). Thus, the present work was aimed at investigating the anti-hyperglycemic and antioxidant actions of the aqueous extract of *Ocimum suave* in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Plant collection and extraction

The leaves of *O. suave* were harvested from gardens around Samaru, Zaria, Nigeria in the month of March. Identification was done at the Herbarium unit, Department of Biological Sciences, Ahmadu Bello University, Zaria. Shade-dried leaves were pulverized and 100 g of the powder was soaked in 1 L distilled water for 24 h, with intermittent shaking. The suspension was filtered first through muslin cloth and then Whatmans' no. 1 filter paper. The filtrate was evaporated to dryness at 45°C and the extract reconstituted in distilled water when required.

Experimental animals

Male Wistar strain rats weighing 120 to 200 g were obtained from the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. They were housed in well ventilated cages and kept in a room where a twelve-hour light/dark cycle was maintained. They were allowed free access to water and fed commercial growers' mash (Vital feeds, Jos) *ad libitum* throughout the period of the experiment. The rats were allowed to acclimatize for two weeks.

Induction of diabetes

Some of the rats were subjected to 8 h fast. Diabetes was induced with a single intraperitoneal injection of alloxan (210 mg/kg body weight). After 72 h, fasting blood glucose levels were measured and 15 rats with fasting blood glucose concentration of more than 150 mg/dl were considered diabetic and included in the experiment.

Experimental design

In this experiment, a total of 25 rats (15 diabetic rats and 10 normoglycemic) were used. The rats were divided into five groups of five rats each. One group of normoglycemic rats was given 0.2 ml/100 g body weight distilled water daily (group 1) by gavage; while the other group of normoglycemic rats (group 2) was similarly given daily oral dose of 800 mg/kg body weight extract. A group of diabetic rats (group 3) was similarly given distilled water as group 1 rats while another (group 4) was treated as group 2 rats. The remaining group of diabetic rats (group 5) was given daily oral dose of 0.08 mg/kg body weight of a standard hypoglycemic drug (Glimenclamide). Weekly body weight change and daily feed-intake were monitored. Fasting blood glucose (using Accu-chek

advantage Glucometer; Roche Diagnostics, USA) and pack cell volume (using Microhaematocrit method) were measured weekly using tail blood. At the end of the 21 days experimental period, the rats were humanely sacrificed in the fasted state. Serum was prepared from blood collected; liver and kidney carefully excised, blotted and weighed.

One gram (1 g) of organ was homogenized in 20 ml 0.01 M phosphate buffer, pH 7.4, and centrifuged at 3,000xg to collect supernatant; which was used as organ extract.

Determination of biochemical parameters

Total cholesterol, high density lipoproteins (HDL)-cholesterol, triacylglycerols, alanine (ALT) and aspartate transaminases (AST) as well as urea were assayed in the serum using commercial reagent kits (Randox Laboratories, UK). Thiobarbituric acid reactive substances assayed as malondialdehyde was determined using method described by Ohkawa et al. (1979). Superoxide dismutase (SOD) activity was assayed by the method described by Misra and Fridovich (1972).

Statistical analysis

Data are presented as means \pm SD and analyzed using analysis of variance (ANOVA) and Duncan post hoc test and significance was determined at $p < 0.05$.

RESULTS

The result of fasting blood glucose (FBG) for all the groups is presented in Figure 1. It showed that the FBG of the two non-diabetic groups remained within the normal range throughout the experimental period. On the other hand, the FBG of all diabetic groups significantly ($p < 0.05$) rose a week after the induction. The diabetic control group maintained their high FBG throughout the period with slight fluctuation. The diabetic group treated with the extract recorded a significant ($p < 0.05$) drop in FBG levels (58%) when compared with that of diabetic control group which recorded a drop of 13% in FBG (Table 1). The blood glucose-lowering effect of the extract was comparable to that of the standard hypoglycemic drug. Diabetes caused a significant ($p < 0.05$) reduction in packed cell volume (Table 2); however, administration of the *O. suave* leaf extract to diabetic rats significantly ($p < 0.05$) prevented the diabetes-induced reduction in packed cell volume; again comparable to the effect of glibenclamide. Induction of diabetes significantly ($p < 0.05$) increased the levels of total -, HDL- and low density lipoproteins (LDL)-cholesterol, and decreased serum triacylglycerols (Table 3). Administration of the extract to diabetic rats kept the levels of serum total -, and LDL-cholesterol significantly ($p < 0.05$) lower than that of the diabetic control animals, but caused further significant increase in the level of serum HDL-cholesterol.

The serum triacylglycerols were returned to near normal values by treatment of diabetic rats with the extract. Induction of diabetes caused a significant

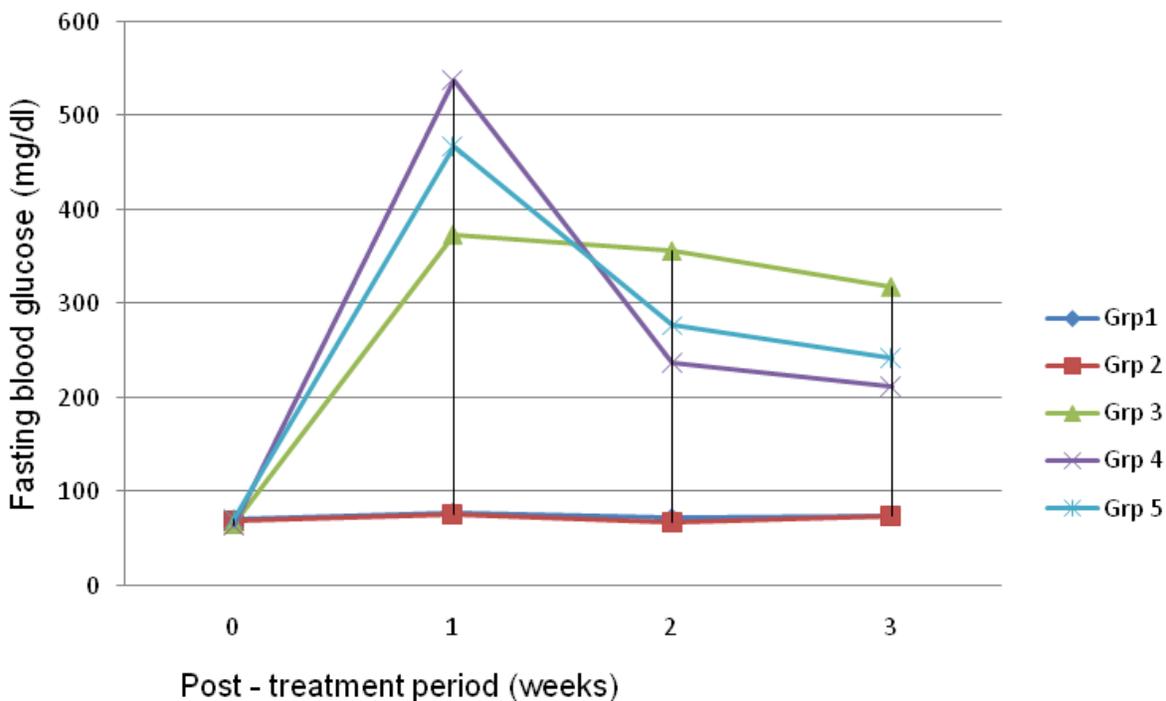


Figure 1. Profiles of fasting blood glucose for all groups.

Table 1. Effect of aqueous leaf extract of *Ocimum suave* on fasting blood glucose (FBG) of normal and alloxan-induced diabetic rats.

Post treatment period	Fasting blood glucose (mg/dl)				
	Group 1	Group 2	Group 3	Group 4	Group 5
Week 1	78.43±9.14	75.80±6.14	373.80±53.37	538.40±88.11	468.67±145.12
Week 3	75.29±9.01	73.67±6.58	318.33±9.42	211.50±12.02	242.50±25.98
*Change in FBG (%)	-15.72±15.94 ^a	-5.91±5.14 ^a	-13.44±13.91 ^a	-58.35±9.15 ^b	-44.06±17.80 ^b

All values are means ± SD of five replicates. Values with different superscripts along a row are statistically different (p<0.05). *These values represent differences between initial and terminal FBG, negative signs indicate decreases. FBG, Fasting blood glucose.

Table 2. Effect of aqueous leaf extract of *Ocimum suave* on packed cell volume (PCV) of normal and alloxan-induced diabetic rats.

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5
Initial PCV (%)	47.00±2.16	46.71± 3.20	49.00±3.10	46.14±3.89	46.57±2.95
Final PCV (%)	42.29±2.69	49.20±4.49	35.33±2.86	42.00±0.71	48.50±1.77
*Change in PCV (%)	-8.68±6.82 ^a	+5.96±11.50 ^a	-27.75±5.47 ^b	-8.46±7.37 ^a	+5.13±6.15 ^a

All values are means ± SD of five replicates. Values with different superscripts along a row are statistically different (p<0.05). *These values represent differences between initial and final PCV, negative and positive signs indicate decreases and increases, respectively. PCV, Packed cell volume.

(p<0.05) increase in serum levels of ALT and a reduction in AST level (Table 3). Treatment with 800 mg/kg of *O. suave* leaf extract did not significantly affect the ALT and AST levels when compared with diabetic control group. All treatments had no significant (p>0.05) effect on the

level of serum urea. Alloxan-induced diabetes caused a significant (p<0.05) increase in levels of thiobarbituric acid reactive substances (TBARS) in kidney and but did not have any effect on level of liver TBARS (Table 4). The levels of TBARS in the liver were not affected by

Table 3. Effect of aqueous leaf extract of *Ocimum suave* on serum lipid profile, urea as well as AST and ALT activities of normal and alloxan-induced diabetic rats.

Parameter (mg/dl)	Group 1	Group 2	Group 3	Group 4	Group 5
Triacylglycerols	57.69±3.47 ^a	74.36±16.70 ^b	30.72±0.04 ^c	77.36±14.20 ^b	74.36±16.32 ^b
Total cholesterol	116.65±16.65 ^a	119.98±16.31 ^a	263.34±25.93 ^b	99.99±23.56 ^c	116.60±11.77 ^a
HDL cholesterol	56.67±9.03 ^a	59.84±10.59 ^a	133.34±16.33 ^b	170.00±35.35 ^c	176.67±21.21 ^c
LDL cholesterol	47.99±9.15	45.27±3.37	123.86±13.57	85.48±20.69	74.95±17.97
Urea	2.91±1.04 ^a	1.31±0.80 ^a	4.79±1.85 ^a	3.20±1.31 ^a	3.40±0.89 ^a
AST (u/l)	15.40±1.57 ^a	23.80±5.14 ^b	8.17±3.78 ^c	8.75±1.24 ^c	8.75±1.24 ^c
ALT (u/l)	12.00±3.89 ^a	32.49±17.20 ^b	27.29±14.06 ^b	25.34±13.79 ^b	33.10±2.81 ^b

All values are means ± SD of five replicates. Values with different superscripts along a row are statistically different (p<0.05). LDL, Low density lipoproteins; HDL, high density lipoproteins; ALT, alanine transaminases; AST, aspartate transaminases.

Table 4. Effect of aqueous leaf extract of *Ocimum suave* on oxidative stress markers in organs of normal and alloxan-induced diabetic rats.

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5
Liver SOD (u/g) X10 ³	6.21±0.44 ^a	6.09±1.19 ^a	6.85±0.57 ^a	1.02±0.13 ^b	5.24±0.23 ^a
Kidney SOD (u/g) X10 ³	6.67±0.55 ^a	0.13±0.01 ^b	4.29±0.16 ^c	0.19±0.01 ^b	2.46±0.11 ^d
Liver TBARS (nmol/g) X10 ⁻³	1.42±1.01 ^a	2.20±0.51 ^a	0.57±0.29 ^{ab}	0.39±0.05 ^b	0.27±0.03 ^b
Kidney TBARS (nmol/g) X10 ⁻³	1.52±0.25 ^a	3.81±0.37 ^b	4.44±0.29 ^b	1.55±0.09 ^a	0.33±0.05 ^c

All values are means ± SD of five replicates. Values with different superscripts along a row are statistically different (p<0.05). SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances.

treatment with the extract or glibenclimide, but in the kidney, the extract caused a significant (p<0.05) decrease in TBARS when compared to diabetic control group. Diabetes also did not affect the level of SOD in the liver, but significantly (p<0.05) reduced the activity of the enzyme in the kidney (Table 4). Treatment of diabetic rats with the extract caused significant (p<0.05) decrease in SOD activities in both liver and kidney.

DISCUSSION

The alloxanised rats in this experiment were all clearly hyperglycemic; an indication of a successful induction of diabetes. The untreated diabetic rats exhibited minimal fluctuations in FBG during the experiment indicating that no reversion to normoglycemic status took place during the period of experimentation. The extract-treated diabetic rats recorded a 58% decrease in FBG, as opposed to the 13% decrease in the diabetic controls. This suggests that the extract had hypoglycemic activity in the diabetic animals; comparable to that of the standard hypoglycemic drug (44%) used in another group of diabetic rats. Reports abound on the presence of anti-hyperglycemic principles in plant preparations (Subramonium et al., 1996; Kim et al., 2011), including the *Ocimum* sp. (Zeggwagh et al., 2007). The anti-hyperglycemic action of the *O. suave* extract seen in this

experiment may be attributed to one or a combination of the following: the active principle(s) may have enhanced the activity of residual insulin in the alloxanised animals or promoted glucose uptake by peripheral tissues, by other means (Twajj and Al-Badr, 1988; Gupta, 1994); it is also possible that the extract slowed down glucose absorption in the gastrointestinal tract (GIT) or regulated the metabolism of glucose by the liver. Dyslipidemia, a feature of diabetes mellitus is found in about 40% of diabetics (Wolfe et al., 2003; Kim et al., 2006), and is directly linked to insulin deficiency (Ramarathnam et al., 1997). Increases in total -, LDL- and HDL-cholesterol were recorded in the untreated diabetic rats in this work, consistent with several earlier reports (Mironova et al., 2000; Barnett and O' Gara, 2003).

Since diabetic dyslipidemia is amongst other factors, a direct consequence of the inability of peripheral tissues to access blood glucose (hence hyperglycemia), any agent that corrects the diabetic hyperglycemia would consequently correct, or at least ameliorate the attendant dyslipidemia. Significant lowering of total cholesterol and rise in HDL-cholesterol is a very desirable biochemical state for prevention of atherosclerosis and ischaemic condition (Fuller et al., 1980). Lipid peroxide-mediated tissue damage has been observed in the development of both types 1 and 2 diabetes mellitus (Stanely et al., 1998). It has been observed that insulin secretion is closely associated with lipoxxygenase derived peroxides

(Gupta, 1994). Wilson et al. (2001) have reported that the concentration of lipid peroxides increased while activities of SOD and catalase reduced in the kidney of diabetic rats. This may result in a number of deleterious effects due to the accumulation of superoxide radicals (O_2^-) and hydrogen peroxide. Similar observations were recorded in the present work. Furthermore, the administration of the extract to diabetic rats caused a reversal to normal levels of the TBARS in the kidney, although, a further decrease in kidney SOD activity was recorded. There was a significant drop in the packed cell volume (PCV) of untreated diabetic rats as compared to the normoglycemic controls. The anemia occurring in DM is due to the increased non-enzymatic glycosylation of red blood cell (RBC) membrane proteins which correlates with hyperglycemia (Twaij and Al-Badr, 1988). Oxidation of these glycosylated membrane proteins in DM causes an increase in the production of lipid peroxides leading to hemolysis of RBCs.

The extract-treated diabetic rats had normal PCV and this may be attributed to both the anti-hyperglycemic and apparent antioxidant activities exhibited by the extract. It is concluded that the aqueous extract of *O. suave* has anti-hyperglycemic and hypolipidemic effects, as well as antioxidant action in alloxan induced diabetic rats.

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