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Full Length Research Paper

Anti-hyperlipidemic effect of aqueous leaf extract of *Ocimum gratissimum* in alloxan induced diabetic rats

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This study aims at evaluating the effect of chronic administration of aqueous leaf extract of *Ocimium gratissimum* on total cholesterol level, triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) in male albino rats. Twenty-one male albino rats of the Wistar strain weighing between 150 and 200 g were randomly assigned into 3 groups of 7 animals per group as control, diabetic and diabetic treated with *O. gratissimum*. Diabetes was induced by 100 mg/kg of alloxan monohydrate. The control and the diabetic groups received distilled water while the diabetic treated group was administered with 400 mg/kg body weight of aqueous leaf extract of *O. gratissimum* for 28 days. At the end of the experiment, plasma glucose level, cholesterol, TG, HDL and LDL were determined in all the experimental animals after 12 h fast. The result showed significant increases (P<0.05) in plasma cholesterol, TG and LDL level of the diabetic group when compared with the control group while there were no significant differences in the OG-treated diabetic group and the control group. The HDL however was not different in all the three groups. It was then concluded that oral administration of aqueous leaf extract of *O. gratissimum* may reduce the plasma lipid imbalances associated with diabetes mellitus.

Key words: *Ocimum gratissimum*, alloxan-induced diabetes, lipid imbalances.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycaemia resulting from a variable interaction of hereditary and environmental factors due to defects in insulin secretion, insulin action, or both (King et al., 1998). It is a disease of epidemic proportion and the number of people developing the disease is growing every year (Ossman, 2006). Rapid growth of diabetes worldwide has been reported and it is envisaged that the number of new cases of diabetes may triple by the year 2030 (Wilde et al., 2004). The disease is ranked as the seventh cause of death in the world and third when its fatal complications are taken into consideration (Trivedi et al., 2004). It is a disease characterized by chronic hyperglycaemia and glucosuria produced by an absolute or relative insufficiency of insulin. The ailment may result in the development of further metabolic further metabolic and anatomic disturbances among which is lipemia, hypercholesterolaemia, loss of weight, ketosis, arteriosclerosis, gangrene, pathologic changes in the eye, neuropathy, renal disease, and coma (Swanston-Flatt et al., 1990; Andrew et al., 2000). Dyslipidaemia is common in diabetes, as both insulin deficiency and insulin resistance affects enzymes and pathways of lipid metabolism (Gibbons, 1988). Dyslipidaemia, as defined by the World Health Organization (WHO, 1994), is considered in circumstances whereby the fasting plasma triglyceride is between 150 and 400 mg/dL (1.7 to 4.5 mmol/L), total cholesterol (TC) > 200 mg/dL (>5.2 mmol/L), low-density lipoprotein (LDL)-cholesterol (LDL-C) > 135 mg/dL (>3.5 mmol/L), high-density lipoprotein (HDL)-cholesterol (HDL-C) < 35 mg/dL (<0.9 mmol/L) in men or <40 mg/dL (<1.0 mmol/L) in women, and a ratio of total cholesterol to HDL-cholesterol > 5 (Jisieike-Onuigbo et al., 2011). Characteristic abnormalities in lipids in type 2 diabetes mellitus include elevated triglycerides (Tg) levels, decreased atheroprotective high density lipoprotein cholesterol (HDL-c) levels,

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and increased levels of small dense LDL-c (Beckman et al., 2002; Navab et al, 1996).

Patients with diabetes mellitus have been treated orally by folklore with a variety of plant extracts (Ajgaonkar, 1979). More than 1200 plants species are used world wide in diabetes phytotherapy, and experimental studies support the hypoglycaemic activity of a large number of these plants (Marles and Farnsworth, 1995). In addition to correction of blood glucose levels, several hypoglycaemic plants have potential in ameliorating lipid metabolism abnormalities of diabetes mellitus (Coon and Ernst, 2003).

Ocimum gratissimum (OG), Africa basil/sweet basil, is a plant belonging to *Lamiceae* family known in Nigeria as *efinrin*, *Nehonwu*, and *ai daya ta guda* by the Yoruba, Igbo and Hausa, respectively. Its major constituents include aromatic and volatile oil, linolenic acid, oleic acid, alkaloid, flavonoid, saponin and cardiac glycosides (Bernardini, 1985; Abdel Barry et al., 2000). It is commonly used in the treatment of upper respiratory tract infection, diarrhoea, fever, conjunctivitis (Celso et al., 1999) and traditional treatment of diabetes mellitus (Bailey and Day, 1989). Recently, its hypoglycaemic efficacy (Aguiyi et al., 2000; Owoyele et al., 2005; Mohammed et al., 2007) and safety (Egesie et al., 2006) have been reported.

Based on the reported activities of most hypoglycaemic plant agents at ameliorating lipid metabolism abnormalities (Thompson Coon and Ernst, 2003), reduction of total cholesterol in normal rats fed with *O. gratissimum* supplemented diet for six months (Iweala and Obidoa, 2010) and no alteration in plasma lipid levels of alloxan-induced diabetic rats following a 7 days treatment with aqueous leaf extract of *O. gratissimum* as reported by Owoyele et al. (2005); it is thus desirable to evaluate the effects of chronic administration of aqueous leaf extract of *O. gratissimum* (OG*)* on the lipid profile of alloxan-induced diabetic rats.

MATERIALS AND METHODS

Preparation of plant material

Fresh leaves of *O. gratissimum* (OG) were collected from Ilorin Metropolis and were taken to the Botany Department, University of Ilorin, Nigeria, for authentication. It was identified with a voucher specimen (FHI, 106934) earlier deposited in the Forestry Research Institute of Nigeria (FRIN) herbarium. The leaves were air dried and reduced to powdered form; the powdered leaves were percolated in distilled water for 12 h and filtered; the filtrate was subsequently evaporated to dryness and yielded a 22.5% dark green concentrate.

Animals

Twenty-one male albino Wistar rats weighing between 150 and 200 g were used for this study. They were housed and acclimatized for two weeks in the Central Animal house of the Faculty of Basic Medical Science, College of Health Sciences, University of Ilorin, Nigeria. They were fed on standard rat pellet diet (Bendel Feeds, Nigeria) and were allowed water *ad libitum*. The animals were maintained under standard laboratory conditions and were subjected to natural photoperiod of 12 h light: dark cycle. All experimental protocols and handling were in compliance with the NIH publication No. 85-23 guidelines (NIH publication revised, 1985).

Induction of diabetes

Diabetes was induced by a single intraperitoneal injection of 100 mg/kg of alloxan monohydrate obtained from Sigma Chemical Co. (St. Louis, MO, USA). Diabetes was confirmed by glucose oxidase method using the glucometer (One Basic, Inc.) after 72 h of alloxan injection, rats with plasma glucose level ≥ 200 mg/dl were separated and used as diabetic in this study.

Experimental design

The rats were randomly distributed into three groups of seven rats each:

Group A – (control) normal and received distilled water
Group B – (untreated) diabetic and received distilled water
Group C – (treated) diabetic and received 400 mg/kg aqueous extract of *O. gratissimum*

The 400 mg/kg dose of aqueous extract of *O. gratissimum* had been reported to be nonlethal (Aguiyi et al., 2000). The rats were treated for four weeks. At the end of the experimental period, they were fasted for 12 h and blood was collected by cardiac puncture under light anaesthesia. The blood was transferred into sample bottles containing EDTA for plasma collection. Plasma was collected from the blood samples after centrifuging at 3000 G for 10 min using a bench centrifuge.

Determination of plasma lipid

Plasma triglycerides and total cholesterol levels were measured using enzymatic colorimetric diagnostic kits obtained from Randox Laboratories, UK. In which the GPO-PAP method of Trinider (1969) was employed. Absorbance was measured at 500 nm. The phosphotungstate precipitation method of Richmond (1973) as applied in Randox kit was used for the determination of HDL-cholesterol. The LDL-cholesterol was estimated using Friedewald (1972) formula:

LDLc = Total cholesterol – HDLc – TG/5

Where LDLc = LDL-cholesterol, HDLc = HDL-choleseterol and TG = triglycerides

Statistical analyses

All results were expressed as mean ± SEM. Data were analyzed by one-way analysis of variance (ANOVA) and Duncan new multiple range test (DMRT). Differences in means were considered significant at $P< 0.05$. All analyses were performed using SPSS 15.

RESULTS

Table 1 shows the mean values of plasma glucose level, total plasma cholesterol, TG, HDL and LDL in the control,

Table 1. Effect of aqueous leaf extract of *Ocimum gratissimum* on plasma glucose level and lipid profile of alloxan induced diabetic rats.

Variable	Control (n=7)	Diabetic untreated (n=7)	Diabetic treated with OG (n=7)
Plasma glucose (mg/dl)	105±4.91	310±14.6	179±8.32**
Total cholesterol (mmol/L)	4.48±0.333	5.81±0.124*	4.65±0.425
Triglycerides (mmol/L)	0.96±0.082	1.54±0.086*	1.20±0.216
HDL (mmol/L)	1.46±0.177	1.17±0.203	1.45±0.241
LDL (mmol/L)	2.83±0.163	3.98±0.050*	3.17±0.228

Values are expressed as mean ±SEM, * P<0.05 when compared to control, **significantly reduced when compared to diabetic untreated group.

diabetic untreated and diabetic treated with *O. gratissimum*.

The total cholesterol level was significantly increased (P<0.05) in the diabetic group when compared with the control group while there was no significant difference in the cholesterol level of the OG-treated diabetic group and the control group. The TG level was significantly increased (P<0.05) in the diabetic group with no significant difference in the OG-treated diabetic group when both were compared with the control group. Though there was no significant difference in the HDL level of the three groups, the diabetic group had a significantly higher (P<0.05) LDL when compared with the control while there was no difference in the LDL level of the OG-treated group and the control group.

DISCUSSION

The increase in the total cholesterol, TG and the LDL levels of diabetic rats observed in this study are in accordance with earlier report documenting increased plasma TG, LDL and cholesterol level in diabetic subject (Oberley, 1988). Diabetes-induced hyperlipidemia has been reported to be attributable to excess mobilization of fat from the adipose due to underutilization of glucose (Nimenibo-Uadia, 2003).

The significant reduction in TG, LDL and cholesterol levels of the diabetic rats when treated with aqueous leaves extract of *O. gratissimum* in this study is in support of the findings that most hypoglycaemic plants have potentials of ameliorating diabetic lipid metabolism anomalies (Coon and Ernst, 2003). This cholesterol lowering effect was earlier reported by Iweala and Obidoa (2010) when used as supplementary diet in normal rats for six months, but contrary to the report of Owoyele et al. (2005) in seven days, a discrepancy was observed which may be due to the difference in the length of administration of the extract.

This hypolipidemic effect of O*. gratissimum* could be related to its chemical composition, which shows the presence of alkaloids, flavonoids, saponin and cardiac glycosides. All these components are known to reduce serum lipid level in animals (Tomita, 1974; Barry, 2000; Ezekwe and Obidoa, 2001). Saponins may lower cholesterol by binding with cholesterol in the intestinal lumen, preventing its absorption, and/or by binding with bile acids, causing a reduction in the enterohepatic circulation of bile acids and increase in its fecal excretion (Rotimi et al., 2011). The increased bile acid excretion is offset by enhanced bile acid synthesis from cholesterol in the liver and consequent lowering of the plasma cholesterol (Beckman et al., 2002).

Though the precise mechanism by which the leave extract exerts it hypolipidemic effect is not clearly known nor studied, it could not be excluded that the control of glycaemia is a contributing mediator since control of glycaemia is a major determinant of total cholesterol and triglyceride levels (Markku, 1995). Accordingly, the evolution of glycaemia was parallel to lipid parameters in both the normal/control and the diabetic rats of this study.

It could thus be concluded that prolonged oral administration of aqueous leaf extract of *O. gratissimum* may reduce the plasma lipid imbalances associated with diabetes mellitus which support its traditional use in the treatment of diabetes and cardiovascular diseases though the precise mechanism(s) and site(s) of action require further elucidation.

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