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Hypoglycaemic and Hypolipidaemic Activities of Solvent Extracts of *Ocimum basilicum* Leaves in Streptozotocin-induced Diabetic Rats

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ABSTRACT
Aqueous, methanolic and petroleum ether extracts of *Ocimum basilicum* leaves at the dose of 200 mg/kg body weight was investigated for hypoglycaemic and hypolipidaemic activities in streptozotocin-induced diabetic rats. Oral administration of 200 mg/kg of the solvent extract for 35 days resulted in a significant reduction ($P<0.05$) in the fasting blood glucose levels of streptozotocin-induced diabetic rats. The extracts at the dose of 2 g/kg body weight enhanced the glucose clearance of the streptozotocin-induced diabetic rats. The extracts significantly ($P<0.05$) reduced the total cholesterol, triglycerides and LDL-cholesterol in all treated groups when compared with the diabetic control with significantly ($P<0.05$) higher values of HDL-cholesterol. The extracts exhibited hypoglycaemic and hypolipidaemic activities in the diabetic rats and hence may be the basis for its folkloric use in the management of diabetic patients in Nigeria.

Keywords: *Ocimum basilicum*, Diabetes, Hypoglycaemic, Hypolipidaemic, Streptozotocin

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of the endocrine system. The disease occurs worldwide and its incidence is increasing rapidly in most parts of the world (Kumar *et al.*, 2008). Diabetes is commonly accompanied by other cardiovascular risk factors such as dyslipidemia, hypertension, prothrombic factors and microvascular problems involving eyes, kidney and peripheral nerves (Grundy *et al.*, 1999; Barnett and O' Gara, 2003). Two types of diabetes are identified based on their clinical manifestations: type 1 diabetes known as juvenile onset or insulin sensitive diabetes and type 2 diabetes or non insulin dependent diabetes mellitus (NIDDM) (Grundy *et al.*, 1999). The latter is the more prevalent. Normally, blood glucose levels are tightly controlled by insulin, but people suffering from diabetes are not able to produce or properly use insulin in the body, so they have a high level of blood glucose.

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Lipids play an important role in the pathogenesis of diabetes mellitus. The level of serum lipids is usually raised in diabetes and such an elevation represents a risk factor for coronary heart disease (Mironava *et al.*, 2000). Lowering of serum lipids levels through dietary or drugs therapy seems to be associated with a decrease in the risk of vascular disease (Scoot and Grundy, 1999). The abnormally high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of fatty acids from the peripheral depots, since insulin inhibits the hormone sensitive lipase. The marked hyperlipidaemia that characterizes the diabetic state may be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots (Al-Shamaony *et al.*, 1994). Dyslipidemia is also characterized by low levels of high-density lipoprotein-cholesterol (HDL-C), and high levels of low density lipoprotein-cholesterol (LDL-C) and triglyceride (TG).

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 (Holman and Turner, 1991). However, DM is also treated in some places using anti-diabetic medicinal plants (Chattopadhyay *et al*., 1993; Ponnachan and Panikkhar, 1993; Subramonium *et al*., 1996).

Ocimum basilicum L, commonly called basil, is a plant belonging to Lamiacea family. In Nigeria, about four species of basil are known to occur naturally in Southern and Northern part of the country. These are *Ocimum suave*, *Ocimum gratissimum*, *Ocimum basilicum* and *Ocimum camium*. Local names of basil amongst various ethic groups include "Efirin" (Yoruba), "neh-anwu" (Ibo), "ntion" (Efik) and "dai-doya ta gida" (Hausa) (Mohammed *et al*, 2007). The phytochemical investigations and elemental analysis of the aqueous leaf extract of *O. basilicum* indicated the presence of pharmacologically useful classes of compounds such as saponin, alkaloids, flavonoids, cardiac glycosides, terpenes, steroids, tannins and carbohydrates (Sanni *et al.*, 2008). The present study thus investigates the effects of extracts of *O. basilicum* on glucose and lipid levels of STZ-induced diabetic rats.

MATERIALS AND METHODS

Chemicals

Streptozotocin (STZ) was a product of Sigma Chemicals, St Louis, U.S.A. All other reagents used were of analytical grade.

Plant material

Fresh leaves of *Ocimum basilicum* were harvested from Enugu, South-East, Nigeria. The plant was identified at the herbarium unit of the Department of Biological Sciences A.B.U, Zaria.

Animals

Forty-five male Wistar strain albino rats weighing between 150-200 g obtained from the Animal House of the Department of Pharmacology, ABU, Zaria, Nigeria were used. The animals were kept under standard laboratory conditions in well ventilated cages. They were maintained on grower's mash (Vital Feeds Nigeria Ltd.) and provided with water *ad libitum*. They were allowed to acclimatize to the laboratory conditions for two weeks before the commencement of the experiment.

Preparation of the solvent extracts

The fresh leaves were collected, washed with clean water and shade dried for six days. The dried leaves were pulverized and used for the subsequent extraction. Aqueous extract was prepared by putting 100 g of the powder in 1 L of distilled water over night. This was then filtered using a filter paper and evaporated to dryness in a water bath maintained at 45^0C. The extract was reconstituted in distilled water when required. For the methanolic extract, 100 g of the powder was extracted in methanol (1 L) for 72 hours at 60^0C using a Soxhlet extractor. The extract was evaporated to dryness. The extract was reconstituted in Tween 80 when required. The petroleum ether extract was done by putting 100 g of the powder in 1.5 L of petroleum ether for 72 hours at 60^0C in a Soxhlet extractor. The extract was also evaporated to dryness and reconstituted in Tween 80 when required.

Acute toxicity test

The LD_{50} for all the different solvent extracts was determined using the method described by Lorke (1983).

Induction of diabetes

Diabetes mellitus was induced by single intraperitoneal dose of 60 mg/kg of streptozotocin, dissolved in 0.1 M fresh cold citrate buffer, pH 4.5; into 12 h-fasted rats (Burcelin *et al.*, 1995). After 3 days, the blood sugar levels were monitored with a glucometer (Acc-Check Advantage, Roche Diagnostics GmbH, Germany) and the rats having fasting blood glucose levels that was greater than 200 mg/dl (11.1mmol/L) were selected for experimentation.

Animal grouping

The rats were assigned into 9 groups of 5 rats each.

Group 1: Diabetic rats treated with 200 mg/kg body weight of the aqueous extract

Group 2: Diabetic rats treated with 200 mg/kg body weight of the methanolic extract

Group 3: Diabetic rats treated with 200 mg/kg body weight of the petroleum ether extract

Group 4: Diabetic rats without any treatment

Group 5: Diabetic rats treated with 2.5 mg/kg body weight of Glibenclamide

Group 6: Non-diabetic rats treated with 200 mg/kg body weight of aqueous extract

Group 7: Non-diabetic rats treated with 200 mg/kg body weight of methanolic extract
Group 8: Non-diabetic rats treated with 200 mg/kg body weight of petroleum ether extract
Group 9: Normal rats (non-diabetic and no treatment).

The administration was done once daily for 35 days with the aid of oral cannula. Fasting blood glucose was monitored on weekly basis. On the 35th day, post-treatment, the animals were fasted overnight, anesthetized with chloroform and sacrificed by humane decapitation. The blood was collected in test tubes. Serum was prepared and used for the various analyses.

Determination of biochemical parameters

The daily feed intake was determined by weighing the initial weight of the feed given to the animals and then the final weight remaining after 24 hours. The actual weight taken by the animals was the difference between the initial weight and the final weight. The fluid intake was determined using calibrated feeding bottles, the initial volume was recorded and the final volume after 24 hours. The actual fluid intake was the difference between the initial and the final volume. The fasting blood glucose levels were determined once a week for the seven weeks of the experimental period with glucometer after fasting the rats for 12hours. For the oral glucose tolerance test (OGTT), rats were fasted for 12 hours and the fasting blood glucose assayed after which the 2g/kg body weight of glucose solution was administered orally. Thereafter, one drop of rats' tail blood was collected at intervals of 30 minutes placed on glucose assay strip and read using glucometer. This lasted for 2 hours. The determinations of the serum total cholesterol, HDL-cholesterol, LDL-cholesterol, were carried out using Randox Assay Kits (Randox, Co-Antrim, UK). Triaylglycerol determination was done using Agappe Assay Kit (Agappe Hills' Kerala, India).

Statistical analysis

The results obtained were statistically analyzed using Analysis of Variance and students' t-test.

RESULTS

The intraperitoneal LD_{50} of aqueous, methanolic and petroleum ether extracts were 470, 3,800 and 3,800 mg/kg body weight respectively. The feed intake in all groups of rats is shown in Figure 1. The results showed that there was significant increase in feed intake in streptozotocin-induced diabetic rats without treatment ($P<0.05$) when compared with the normal control group. Treatment with extracts or reference drug (glibenclamide) reduced the feed-intake to near normal levels. The A significant increase in the fluid intake ($P<0.05$) was observed in diabetic control group when compared with the normal control group (Figure 2). Treatment with the extracts or glibenclamide significantly reduced the fluid-intake of diabetic rats; but not to the levels of non-diabetic rats.

The non-diabetic rats showed little or no change in body weight throughout the experiment; but the untreated diabetic rats produced a consistent and progressive decrease in body weight. The diabetic rats treated with either the extracts or glibenclamide exhibited weight recovery by the second week of the experiment. At the end of the experiment, only the untreated diabetic group produced a final body weight that was significantly ($P<0.05$) lower than the initial (Figure 3).

All the non-diabetic rats, whether treated or not, maintained normoglycemic blood glucose levels throughout the period of experimentation (Figure 4). The fasting blood glucose of all diabetic groups was initially at hyperglycaemic levels on day 0. For the diabetic control, the fasting blood glucose levels remained at diabetic levels throughout the duration of the experiment. All diabetic groups treated with the extracts or glibenclamide exhibited significant ($P<0.05$) decrease in the fasting blood glucose level. By the 35th day, the diabetic control animals produced a 30.24% increase in fasting blood glucose from the initial value, while diabetic rats treated with the glibenclamide and methanolic extract produced 73.77% and 77.88% decreases in fasting blood glucose when compared to their initial values, respectively (Table 1).

The treated diabetic rats and normal control rats recorded normal glucose tolerance while the diabetic control rats

showed impaired glucose clearance (Figure 5).

There were significant increases (P<0.05) in the level of total cholesterol, triacylglycerol and LDL-cholesterol in diabetic control group when compared with the non-diabetic groups (Table 2). There was a significant decrease (P<0.05) in the level of HDL-cholesterol in diabetic control group compared with that of non-diabetic rats. Treatment with extracts of *Ocimum basilicium* leaf caused significant decreases (P<0.05) in LDL-cholesterol, total cholesterol and triacylglycerol as well as a significant increase in HDL-cholesterol from diabetic levels recorded in the diabetic control group. These effects were comparable to those of the diabetic animals treated with glibenclamide (Table 2).

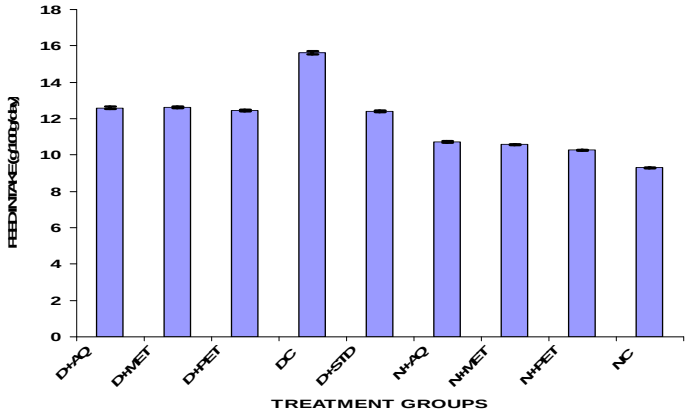

Fig. 1: Daily feed intake of diabetic and non-diabetic rats treated with extracts of *O. basilicum* leaf

D+AQ: Diabetic rats + Aqueous Extract, D+MET: Diabetic rats + Methanolic Extract, D+PET :Diabetic rats + Petroleum Ether, DC: Diabetic rats Control D+STD: Diabetic rats + Standard Drug, N+AQ: Normal rats + Aqueous Extract, N+MET: Normal rats + Methanolic Extract , N+PET: Normal rats + Petroleum Ether and NC: Normal rats Control.

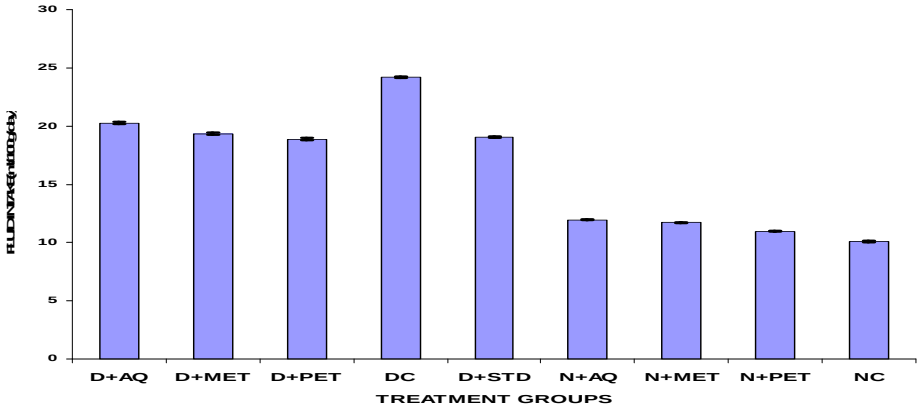

Fig. 2: Daily fluid intake of diabetic and non-diabetic rats treated with extracts of *O. basilicum* leaf

D+AQ: Diabetic rats + Aqueous Extract, D+MET: Diabetic rats + Methanolic Extract, D+PET :Diabetic rats + Petroleum Ether, DC: Diabetic rats Control D+STD: Diabetic rats + Standard Drug, N+AQ: Normal rats + Aqueous Extract, N+MET: Normal rats + Methanolic Extract , N+PET: Normal rats + Petroleum Ether and NC: Normal rats Control

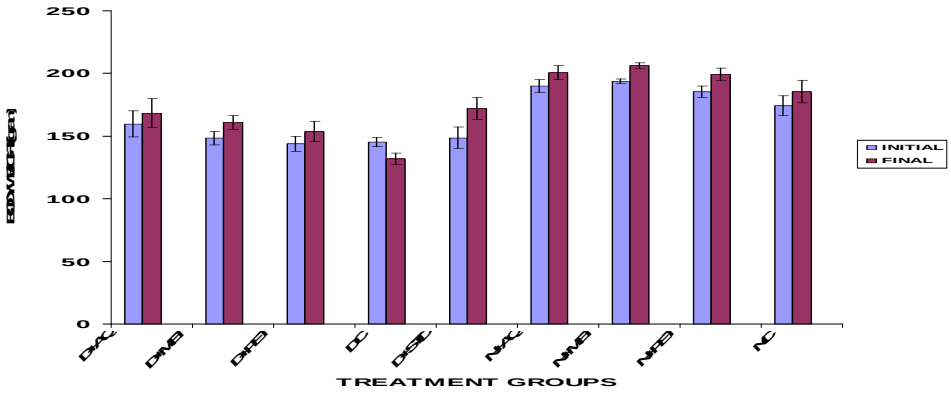

Fig. 3: Body weights of diabetic and non-diabetic rats treated with extracts of *O. basilicum* leaf

D+AQ: Diabetic rats + Aqueous Extract, D+MET: Diabetic rats + Methanolic Extract, D+PET :Diabetic rats + Petroleum Ether, DC: Diabetic rats Control D+STD: Diabetic rats + Standard Drug, N+AQ: Normal rats + Aqueous Extract, N+MET: Normal rats + Methanolic Extract , N+PET: Normal rats + Petroleum Ether and NC: Normal rats Control

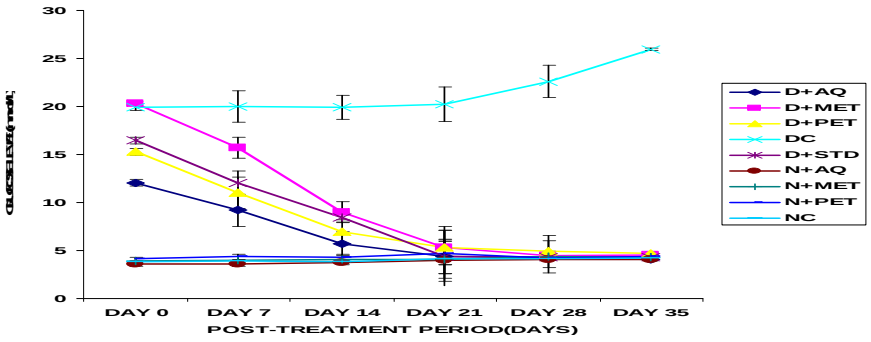

Fig. 4: Fasting blood glucose of diabetic and non-diabetic rats treated with extracts of *O. basilicum* leaf

D+AQ: Diabetic rats + Aqueous Extract, D+MET: Diabetic rats + Methanolic Extract, D+PET :Diabetic rats + Petroleum Ether, DC: Diabetic rats Control D+STD: Diabetic rats + Standard Drug, N+AQ: Normal rats + Aqueous Extract, N+MET: Normal rats + Methanolic Extract , N+PET: Normal rats + Petroleum Ether and NC: Normal rats Control.

Table 1: Percentage change in fasting blood glucose of diabetic and non-diabetic rats extracts of *O. baslicum* leaf or Glibenclamide

GROUP	D+AQ	D+MET	D+PET	DC	D+STD	N+AQ	N+MET	N+PET	NC
Change in Fasting blood Glucose (%)	-66.11± 3.23[a]	-77.88± 6.77[a]	-69.15± 4.39[a]	30.24± 3.96[c]	-73.77± 5.25[a]	14.53± 0.39[b]	10.31± 0.32[b]	6.81± 0.37[b]	10.47± 0.48[b]

Values are means ±SD of five replicate determinations. Values with different superscript (a, b, c) in the same row are statistically different (P<0.05)

D+AQ: Diabetic rats + Aqueous Extract, D+MET: Diabetic rats + Methanolic Extract, D+PET :Diabetic rats + Petroleum Ether, DC: Diabetic rats Control D+STD: Diabetic rats + Standard Drug, N+AQ: Normal rats + Aqueous Extract, N+MET: Normal rats + Methanolic Extract , N+PET: Normal rats + Petroleum Ether and NC: Normal rats Control.

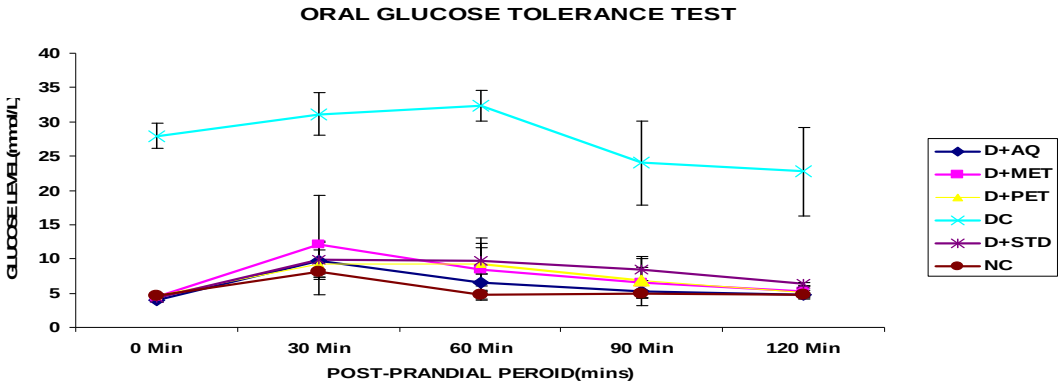

Fig. 5: Oral glucose tolerance of diabetic and non-diabetic rats treated with extracts of *O. basilicum* leaf

D+AQ: Diabetic rats + Aqueous Extract, D+MET: Diabetic rats + Methanolic Extract, D+PET: Diabetic rats + Petroleum Ether, DC: Diabetic rats Control, D+STD: Diabetic rats + Standard Drug and NC: Normal rats Control.

Table 2: Effects of extracts of *Ocimum baslicum* leaf or Glibenclamide on HDL-cholesterol, LDL-cholesterol, total cholesterol and triglycerides in diabetic and non-diabetic rats

Lipid parameters (mg/dl)	D+AQ	D+MET	D+PET	DC	D+STD	N+AQ	N+MET	N+PET	NC
HDL-cholesterol	93.33 ±2.78^{c}	92.12 ±4.37^{c}	93.46 ±2.80^{c}	38.70 ±2.66^{b}	94.46 ±3.40^{c}	81.97 ±2.29^{b}	80.46 ±2.71^{b}	80.28 ±3.41^{b}	83.05 ±9.37^{b}
LDL-cholesterol	86.17 ±3.55^{bc}	101.02 ±8.73^{d}	95.00 ±2.80^{cd}	280.79 ±23.79^{e}	105.75 ±4.85^{d}	65.53 ±4.20^{a}	66.89 ±3.76^{a}	66.30 ±4.26^{a}	75.32 ±8.46^{ab}
Total cholesterol	97.74 ±4.12^{b}	113.68 ±3.85^{c}	101.37 ±3.85^{b}	391.66 ±5.57^{d}	118.76 ±2.83c	55.52 ±4.17a	60.83 ±1.99^{a}	56.30 ±2.08^{a}	61.76 ±4.48^{a}
Triglycerides	71.12 ±10.65^{a}	71.85 ±6.84^{a}	69.22 ±5.91^{a}	221.12 ±15.42^{b}	70.39 ±4.29^{a}	66.60 ±7.04^{a}	65.53 ±7.44^{a}	63.50 ±6.89^{a}	67.67 ±6.04^{a}

Values are means ±SD of five replicate determinations. Values with different superscript (a, b, c, d, e) on the same row are statistically different (P<0.05)

D+AQ: Diabetic rats + Aqueous Extract, D+MET: Diabetic rats + Methanolic Extract, D+PET :Diabetic rats + Petroleum Ether, DC: Diabetic rats Control D+STD: Diabetic rats + Standard Drug, N+AQ: Normal rats + Aqueous Extract, N+MET: Normal rats + Methanolic Extract , N+PET: Normal rats + Petroleum Ether, NC: Normal rats Control

DISCUSSION

In this study, the streptozotocin-induced rats without treatment showed significantly higher levels of fasting blood glucose, polyphagia, polydypsia and lower body weight compared to normal control and diabetic rats treated with the different extracts. These were consistent with earlier reports (Urmila and Goyal, 2003), and are established features of diabetes. The present study has established the hypoglycaemic and hypolipidaemic activities of aqueous, methanolic and petroleum ether extracts of *Ocimum basilicum* leaf in normal and streptozocin-induced diabetic rats. In the present investigation, daily administration of aqueous, methanolic and petroleum ether extracts of *Ocimum basilicum* leaf resulted in decrease in blood glucose levels in STZ-induced diabetic rats. Though all the three extracts proved effective in this study, the methanolic extract had the highest capacity to restore fasting blood glucose to near normal levels. In a previous report by Ojewole (2003), on *Anacardium occidentale*, the hypoglycemic effect of the methanolic plant extract was found to be slightly more pronounced than that of the aqueous plant extract in both the normal and diabetic rats examined. The possible mechanism by which extracts of *Ocimum basilicum* leaf brings about its hypoglycaemic action may be by potentiating insulin effect of plasma by increasing either the pancreatic secretion of insulin from *ß*-cells of islet of Langerhans that may not have been destroyed by the diabetogen . It also may be that the extracts increased the rate of uptake of glucose in blood, regulate the absorption of glucose in the gastrointestinal tract (GIT) or regulate the metabolism of glucose in liver. In this context, a number of other plants have also been observed to have hypoglycaemic and insulin-release stimulatory effects (Twaij and Al-Badr, 1988; Gupta, 1994).

It has been established that prolonged garlic feeding ultimately causes reduction in the level of reduced glutathione form (G-SH) (Umar *et al.*, 1996; 1998). Reduced glutathione and albumin rich in –SH groups participate in the degradation of insulin (Jain and Vyas, 1975; Chang and Johnson, 1980). Reduction in the levels of G-SH and other SH group proteins would lead to reduced degradation of insulin hence increasing the half-life of circulatory insulin (Umar *et al.*, 2003). In this present study, the STZ-induced diabetic rats may have contained some residual insulin activity. It may be that the extracts have a way of increasing the half-life of insulin which could be of help in management of diabetes mellitus.

Oral glucose tolerance test is a series of blood glucose tests done to diagnose diabetes. In a non-diabetic individual, the glucose levels rise and then fall quickly and in diabetic patient the glucose levels rise higher than normal and fail to come back down fast. In OGTT of this study, aqueous, methanolic and petroleum ether extracts of *Ocimum basilicum* leaf caused significant reduction in the serum glucose levels of the animals. It was observed that glucose clearance in treated diabetic groups was similar to normal control group and diabetic control group showed impaired clearance. Based on the hypoglycaemic effects in normal and diabetic rats, it could be proffered that the hypoglyaemic mechanism involved an insulin-like effect, most probably through the peripheral glucose consumption (Laakso, 1999).

It is also noteworthy in the present study that when extracts of *Ocimum basilicum* leaf was administered to streptozotocin-induced diabetic rats, weight loss was reversed and rats returned to near normal. A similar effect was also observed by Twaij and Al-Badr, (1988). The ability of *Ocimum basilicum* leaf to prevent weight loss seems to be due to its ability to reduce hyperglycaemia. In this study, the significant reduction in food and water intake in extract-treated streptozotocin induced diabetic rats is noteworthy. This could be the result of improved glycemic control produced by the extracts of *Ocimum basilicum* leaf.

Abnormalities in lipid profile are one of the most common complications in diabetes mellitus, which is found in about 40% of diabetics (Stanely *et al.*, 1998). Insulin deficiency is associated with hypercholesterolemia, and hypertriglyceridemia (Gupta, 1994). Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue. This results in increased production of cholesterol-LDL particles (Ramarathnam *et*

al., 1997). Insulin deficiency leads to a fall in lipoprotein lipase activity. Hypertriglyceridemia is also associated with metabolic consequences of hypoinsulinemia, insulin resistance and glucose intolerance (Reaven *et al.*, 1993). 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). This study showed that the extracts were effective in decreasing the serum lipids. Previous studies have demonstrated that in addition to the hypoglycaemic activity, several plants posses plasma lipid lowering activity in animal model of diabetes mellitus (Twaji and Al-Badr, 1988). This hypolipidemic effect may be due to an increase in insulin secretion that leads to a decrease in the synthesis of cholesterol and fatty acid.

The study showed that treatment with various extracts of *Ocimum basilicum* leaf did not induce weight gain in the rats when compared with standard drug (glibenclamide), which is a major advantage over some synthetic hypoglycemic agents. The extracts exhibited hypoglycaemic and hypolipidaemic activities which reduced cardiovascular risk factors. Though the three extracts proved effective as hypoglycaemic agents, the methanolic extract had the highest capacity to reduce glucose. These results support its traditional use in the management of diabetes and cardiovascular diseases.

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