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Protective effects of virgin olive oil on hyperglycaemia and dyslipidaemia in streptozotocin-induced diabetic rats

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ABSTRACT

The main aim of the present study was to investigate the effects of virgin olive oil (VOO) on streptozotocin (STZ) - induced hyperglycaemia and dyslipidaemia in rats. For this purpose experimental diabetes was induced by a single intraperitoneal injection of STZ (60 mg/kg). VOO (0.25ml/kg and 0.5 rnl/ kg) was orally administered to diabetic rats for a period of 30 days. Significant increase in blood glucose, total cholesterol (TCh), triglycerides (TG), low densitylipoprotein (LDL) and very low density lipoprotein (VLDL) cholesterol levels with significant decrease in high density lipoprotein (HDL) cholesterol levels were observed in diabetic control rats. Oral administration of VOO to diabetic rats significantly decreased the elevated levels of blood glucose. The most interesting finding was the significant increase in HDL.-cholesterol levels whereas, there was a significant decrease in serum TCh, TG, LDL and VLDL cholesterol levels in VOO treated diabetic rats. These results suggested that VOO effectively prevented the hyperglycaemia and improved the overall lipid profile of diabetic rats. These results obtained clearly indicate the existence of abnormalities in glucose and lipid metabolism in STZ diabetic rats and suggest a protective effects of VOO in this animal model.

Key words: *Diabetes mellitus*, Virgin olive oil, Streptozotocin, Hyperglycaemia, Dyslipidaemia.

INTRODUCTION

Virgin olive oil is fruit oil obtained from the olive (*Olea europea*; Family Oleaceae). It contains triacylglycerols and small quantities of free fatty acids, glycerol, pigments, aroma compounds, sterols, tocopherols, phenols, unidentified resinous components and others. The antioxidants phytochemicals present in VOO are hydroxytyrosol and oleuropein. VOO, as a highly monounsaturated oil and is resistant to oxidation. Evidence from epidemiological studies suggests that a higher proportion of monounsaturated fats in the diet is linked with a reduction in the risk of coronary heart disease (Carluccio *et al.,*2007). Also the presence of phenols, tocopherols and other natural antioxidants prevent lipid oxidation within the body eliminating the formation of free radicals which may cause cell destruction. The aroma and the flavour compounds of VOO, as well as the chlorophyll and pheophytin pigments, increase the stomach secretion and facilitate the absorption of the natural antioxidants, which furthermore protect the body tissues from oxidation. Possibly due to its antioxidant property VOO has been shown to protect different organs and cells against a number of insults. The effects of VOO on hyperglycemia and dyslipidaemia in STZ - induced diabetes, however have not yet been studied. We therefore investigated the effects of VOO on STZ - induced hyperglycaemia and dyslipidaemia Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycaemia associated with absolute or relative deficiencies in insulin secretion or function. Diabetes is possibly the world's fastest growing metabolic disease; it is the third commonest disease in the world only next to the cardiovascular and oncological disorders according to World Health Organization data. DM

produces lipid abnormalities, which isthe main risk factor for coronary heart disease (Biesbroeck *et al.,*1982; Krolewski *et al.,*1987). In both types of diabetes, abnormalities of lipid metabolism are important, but the nature of these abnormalities is different and the therapeutic approach also differs.

Elevated triglyceride (TG) level, reduced high density lipoprotein (HDL) cholesterol and a preponderance of small, dense low density lipoprotein (LDL) particles are the key abnormalities that Constitute diabetic dyslipidaemia Barrett-Connor *et al.,*1982). It has long been known that people with diabetes are more likely to have cardiovascular risk factors including obesity, dyslipidaemia and hypertension. Early detection and treatment of elevated cholesterol levels is likely to be of benefit in these patients (Pyolara *et al.,*1997).

MATERIAL AND METHODS

Drugs and chemicals

Streptozotocin (STZ) was purchased from the Sigma Chemicals Co. (USA). Virgin olive oil was obtained from Alkoms – Libya . Glucose, cholesterol and triglycerides kits were purchased from the Span Diagnostics Ltd . All the other chemicals used were of analytical grade.

Animals

Male Albino rats weighing (150-200 g) were used for this study. They were kept in the animal house (Faculty of Medicine, 7th October University, Misurata, Libya) for one week. food and water were allowed ad labium throughout the experimental period.

Experimental design

The rats were divided into four groups containing six animals in each group and receivedthe following treatment ´ Group 1: Normal control rats, received normal saline (I ml/ kg, p.o) for 30 days

- Group II: Diabetic control rats, received STZ in a single dose (60 mg/kg, i.p)
- Group III: Diabetic treated rats, received olive oil (0.251-ml/ kg, p.o) for 30 days
- Group IV: Diabetic treated rats, received olive oil (0.5ml/kg, p.o) for 30 days.

Induction of diabetes

STZ in citrate buffer (pH-4.5) was administered intraperitoneally (i.p.) at a single dose of 60 mg/kg to groups 11, 111 and IV. Three days after STZ treatment, development of diabetes was confirmed by measuring blood glucose level. Rats with blood glucose levels of 200 mg/dl or higher were considered to be diabetic. Three days after the STZ treatment VOO (0.25ml/kg and 0.5 ml/ kg, p.o) was given and this was continued daily until the end of the study (30 days) to group III and IV rats.

Biochemical estimations

On the last day of experiment, blood samples were collected for biochemical estimations. Blood glucose was determined by glucose oxidase (Braham and Trinder 1972) method using a commercial diagnostic kit from Span. Serum was separated for assessment of lipid profile i.e. serum cholesterol (Warnick *et al.,*1985), serum triglycerides (Bucolo and David 1973) were estimated using diagnostic kits. LDL and VLDLcholesterol levels were calculated by Friedwald method (Friedewald *et al.,*1972).

Statistical analysis

Data were expressed as the mean ± standard error (S.E) of the means. For a statistical analysis of the data, group means were compared by one-way analysis of variance (ANOVA) with post hoc analysis. The Tukey-Karmer test post hoc was

RESULTS

Table 1 shows the effects of VOO on blood glucose levels. Significant ($P < 0.001$) increase in the blood glucose levels were observed in animals treated with STZ when compared with normal control rats. Oral administration of VOO at two doses (0.25ml/kg and 0.5 ml/ kg) significantly reduced the blood glucose levels ($P < 0.001$). Higher dose (0.5ml/kg) showed better protective effect in reducing elevated blood glucose levels. Table 2 and 3 shows the effects of VOO on the serum lipid profile. A significant increase ($P < 0.001$) in TCh, TG, LDL and VLDL-cholesterol levels were observed in STZ diabetic rats. Administration of VOO at the doses of (0.25ml/kg and 0.5 ml/ kg) significantly ($P <0.001$) reduced the elevated levels

Table 1: Effect of VOO on blood glucose levels in STZ - induced diabetic rats

Groups	Treatment	Blood Glucose mg/dl
I	Normal control (Saline; 1 ml/kg, p.o)	93.16+5.21
II	Diabetic control (STZ; 60 mg /kg, i.p)	298.02±1 1.31++
III	Diabetic + Virgin olive oil (0.25 ml/kg, p.o)	142.51±6.32**
IV	Diabetic + Virgin olive oil (0.5 ml/kg P.0)	11 5.89±6.91

The data are expressed in mean ± SEM; n=6 in each group. ++ P < 0.01 compared with the corresponding value for normal control group. ** P < 0.01 compared with the corresponding value for diabetic control group.

Table 2: Effect of VOO on serum lipid profile (Total & HDL cholesterol, Triglycerides) in STZ - induced diabetic rats

Groups	Treatment	Serum T Chmg/dl	Serum HDLmg/dl	Serum TGmg/dl
I	Normal control (Saline; I ml/kg, p.o)	60.45+2.73	40.34+1.30	69.79+2.92
II	Diabetic control (STZ; 60 mg /kg, i.p)	152.02+6.20	26.76±0.34"	182.50+7.30+4
III	Diabetic + Virginolive oil (0.25 ml/kg,P.0)	89.20+2.20**	33.45+0.70**	103.63±4.33**
IV	Diabetic + Virginolive oil (0.5 ml/kg,	78.89+2.28**	38.21+0.94**	83.47+3.71**

The data are expressed in mean ± SEM; n=6 in each group. ++ P < 0.01 compared with the corresponding value for normal control group. ** P < 0.01 compared with the corresponding value for diabetic control group.

Table 3: Effect of VOO on serum lipid profile (LDL and VLDL -Cholesterol) in STZ . induced diabetic rat

Groups	Treatment	Serum LDLmg/dl	Serum VLDLmg/dl
	Normal control (Saline; 1mllkg, p.o)	38.42+0.67	13.95±0.32
11	Diabetic control (STZ; 60 mg /kg, i.p)	110.16+3.78**	36.5+1.64**
	Diabetic + Virgin olive oil (0.25 ml/kg, p.o)	61.38±1.69"	21.12±0.85'-'
IV	Diabetic + Virgin olive oil (0.5 ml/kg, p.o)	54.551-1.13'-'	16.69±0.63+

The data are expressed in mean ± SEM; n=6 in each group. ' P < 0.01 compared with the corresponding value for normal control group. ** P < 0.01 compared with the corresponding value for diabetic control group.

of these cholesterols and triglycerides when compared with diabetic control rats. Diabetic animals treated with VOO (0.5 ml/kg) had better effect in respect to the reduction in the level of serum cholesterol and triglycerides. The HDL-cholesterol level was decreased significantly ($P < 0.001$) in STZ diabetic rats when compared with normal control rats. VOO in two doses increased the HDLcholesterol levels significantly ($P < 0.001$). Diabetic rats treated with VOO (0.5 ml/kg) had almost same levels of HDL cholesterol as of normal animals.

DISCUSSION

Cardiovascular diseases are the leading cause of mortality in patients with diabetes (Stamler *et al.,*1993). The long-term complications of diabetes are of great concern, but cardiovascular diseases are particularly serious. This accounts for much premature morbidity and mortality and is responsible for about 80% deaths among patients with type 2 diabetes (Barnett 2001). For this reason, health professionals are emphasizing the importance of managing not just glycemia, but also other cardiovascular risk factors including obesity, dyslipidaemia and hypertension. yslipidaemia, which may include raised triglycerides, total cholesterol, LDL-cholesterol, VLDL-cholesterol and low HDL-cholesterol levels seen in well over a third of people with diabetes and was observed in our study on STZ-diabetic rats.

Previous studies have shown that olive phenolics are powerful antioxidants and could partially account for the observed health benefits of the Mediterranean diet (Fito *et al.,*2007). The main phytochemicals for this antioxidant action are hydroxytyrosol and oleuropein. The intake of oleuropein may help in the prevention of diabetic complications associated with oxidative stress. Invitro studies have also shown that oleuropein is non-toxic antioxidant with potent anti-tumor effects. Epidemiological data also showed that the Mediterranean people who consume a lot of olive oil have low rates of cardiovascular disease (Martinez-Gonzalez *et al.,*2002), cancer of the breast and of high life expectancy (Escrich *et al.,*2007). In recent studies of in-vitro and ex-vivo models, olive oil phenolics have shown to have antioxidant properties, higher than that of vitamin E, on lipids and DNA oxidation (Own *et al.,*2000; Fit *et al.,*2000). Olive oil is a priceless source of vitamins and polyphenolic antioxidants, and has a balanced ratio of monounsaturated and polyunsaturated fatty acids. There are multiple mechanisms by which olive oil might impact the development of atherosclerosis. Olive oil decreases LDLcholesterol and increases HDL-cholesterol, and also reduces oxidative stress due to polyphenols, which are able to scavenge free radicals and protect LDL from oxidation. In addition, olive oil components may interfere with the inflammatory response within atherosclerotic lesion, by inhibiting endothelial activation involved in monocyte recruitment during early atherogenesis and macrophage production of inflammatory cytokines and matrix degrading enzymes, thus improving vascular stability (Carluccio *et al.,* 2007).

Diabetic control rats showed persistent hyperglycemia. VOO treated diabetic rats however significantly reduced the blood glucose levels thereby showing its antihyperglycemic activity. It is corresponding value for diabetic control group. not known whether the antihyperglycemic activity is due to increased insulin production and release or improvement in the insulin sensitivity. The precise mechanism by which VOO shows ant hyperglycemic activity is still being probed. In the present study when VOO was given to diabetic rats, the serum TCh, TG, LDL-cholesterol and VLDL-cholesterol levels were significantly reduced, in addition to a reduction of blood glucose levels. We observed an increase in HDL-cholesterol levels in VOO treated rats. This finding is in favour of olive oil, since low HDL-cholesterol levels are considered as a risk for coronary heart disease. HDL is strongly protective against atherosclerosis. An important mechanism underlying this protective effect is the role of HDL in the removal of excess cholesterol from the peripheral tissues. But in addition, HDL also protects by inhibiting lipoprotein role for oxidation of lipoproteins such as LDL in the pathogenesis of atherosclerosis (Steinberg *et al.,*1989; Witztum and Steinberg 1991). Over the past decade, a number of promising new targets have been identified that presents exciting new

opportunities for treatment of low VLDL levels. It has also become clear that HDL. is functionally very heterogeneous (Navab *et al.,*1996). Thus, rather than attempting to increase levels of HDL, it may be more productive to focus on functional properties such as its antioxidant activity. Olive oil itself an antioxidant was found to improve the HDL levels indiabetic rats.

In conclusion , the present study demonstrated the protective effects of olive oil in restoring the blood glucose levels to almost near normal levels and improving the lipid profile in diabetic rats. These results suggest that olive oil may be therapeutically beneficial for diabetes patients with dyslipidaemia

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