

Antioxidant and Hypolipidemic Effects of Olive Oil in Normal and Diabetic Male Rats*

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Abstract

Diabetes mellitus manifests itself in a wide variety of complications and the symptoms of the disease are multifactorial. The lipid hydroperoxide level and lipid profile were investigated in plasma of normal and Alloxan-induced diabetic rats treated with olive oil for six weeks. Diabetic rats exhibited an increase in the levels of hydroperoxide, cholesterol, triglycerides and low density lipoprotein (LDL), and a decrease in the level of high density lipoprotein (HDL). The administration of olive oil showed a better profile in the lipid as well as decreases in the concentration of lipid hydroperoxides either in normal or diabetic rats. The results are discussed according to antioxidant property of olive oil.

Key Words: Olive oil, Diabetes, Lipid profile, Antioxidant.

Introduction

Oxidative stress generated by hyperglycemia and hyperlipidemia is regarded as an important mediator of diabetic complications. The presence of free radicals and the simultaneous decline of antioxidant defense mechanisms observed in diabetic patients could promote the development of diabetic complications (Godin *et al.*, 1988).

Alloxan has been proposed to act as a diabetogenic agent due to its ability to destruct pancreatic β -islets cells, possibly by free radical mechanism. Diabetes represents a state of increased lipid peroxidation and reduced antioxidant reserve (Panneerselvam and Govindasamy, 2004).

Olive oil is one of the main sources of dietary fatty acids. Olives and their oil contain oleic acid and a series of polyphenols (Montedoro *et al.*, 1992) which have been shown in studies *in vitro* to inhibit platelet function (Petroni *et al.*, 1995), and to stimulate the uptake of free radicals by leukocytes (De La Puerta *et al.*, 1999). Epidemiological evidence shows that the Mediterranean diet containing olive oil is associated with lower incidence of both coronary heart disease and certain tumors

(Visoli and Galli, 1998). Moreover, olive oil is rich in monounsaturated and low in saturated fatty acids.

This study evaluates the antioxidant activity of olive oil in normal and alloxan-induced diabetic rats. Moreover, the role of olive oil in lipid profile change was investigated.

Materials and methods

Animals

Fourty male albino rats weighting between 160 and 180 g. were procured from Pharmacy College, King Saud University, Saudi Arabia. The animals were housed in a well ventilated 12 h light and dark cycle and maintained on a commercial rat ration. The animals were divided into 4 equal groups: group I, normal control, group II normal rats orally administered with olive oil (0.5 ml/100 g) through gastric tube for 45 days, group III, alloxan treated animals (150 mg/kg intraperitoneally) and group IV, diabetic rats treated with olive oil as in normal group.

Blood sampling

Blood was collected after three days from alloxan treatment and this is considered zero time. Moreover, blood was taken at 2, 4 and 6 weeks from eyes of all

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groups in heparinized tubes. Plasma was separated and kept in freezer till the time of assay.

Biochemical analysis

The following analyses were carried out: Glucose, Cholesterol, Triglycerides, Low and high density lipoprotein using kits from *Bio Merieux*, France. Blood hydroperoxide level was evaluated using free radical analytical system (IRAM, PARMA, Italy). The test is a colorimetric test that takes advantage of the ability of hydroperoxides to generate free radicals after reacting with some transitional metals. When buffered chromogenic substance is added, a coloured complex appears.

Statistical analysis

Statistical difference was calculated by using the student T-test.

Table 1. Glucose concentration (mg/100 ml) in plasma of normal and diabetic rats treated with olive oil.

Time in week	Normal rats		Diabetic rats	
	Control	Olive oil	Control	Olive oil
0	93.15 ± 5.03	81.41 ± 6.14	290.00 ± 9.41*	314.00 ± 15.08*
2	89.36 ± 3.11	90.56 ± 1.83	308.17 ± 11.53*	292.94 ± 3.85*
4	98.35 ± 6.91	93.14 ± 2.63	312.36 ± 12.61*	275.93 ± 9.51*
6	90.00 ± 2.53	85.46 ± 3.10	298.43 ± 13.00*	247.50 ± 10.21**

Each value is the mean ± S.E., n = 10.

* : Significantly different from corresponding normal group at each time interval, p<0.01.

** : Significantly different from diabetic control, p<0.01.

Table 2. Blood hydroperoxide level (Carr unit) in normal and diabetic rats treated with olive oil.

Time in week	Normal rats		Diabetic rats	
	Control	Olive oil	Control	Olive oil
0	323.00 ± 29.06	306.26 ± 15.30	430.18 ± 11.28*	415.33 ± 17.16*
2	302.11 ± 15.38	295.46 ± 17.42	484.40 ± 26.93*	423.53 ± 18.97*
4	315.60 ± 22.00	280.75 ± 13.47	495.82 ± 13.15*	430.53 ± 12.00**
6	300.95 ± 15.71	260.14 ± 13.30♦	492.16 ± 10.06*	439.35 ± 16.34**

Each value is the mean ± S.E., n = 10.

* : Significantly different from corresponding normal group at each time interval, p<0.01.

♦ : Significantly different from normal control, p<0.05.

** : Significantly different from diabetic control, p<0.01.

Results

1. Glucose concentration

Table 1, depicts the level of plasma glucose in control and experimental rats. Diabetic rats showed marked elevation in glucose concentration in all time intervals. Olive oil has no significant influence on plasma glucose level of normal rats, while it decreased significantly (p<0.01) in the diabetic rats treated with olive oil after six weeks.

2. Hydroperoxide level

As shown in table 2, the blood hydroperoxide level increased significantly (p<0.01) in diabetic rats as compared to normal ones. Olive oil treatment significantly (p<0.05) lowered the hydroperoxide level in normal rats after 6 weeks. Moreover, the oil significantly (p<0.01) lowered the concentration of peroxide in diabetic rats after 4 and 6 weeks as compared to diabetic control.

Table 3. Plasma cholesterol concentration (mg/100 ml) in normal and diabetic rats treated with olive oil.

Time in week	Normal rats		Diabetic rats	
	Control	Olive oil	Control	Olive oil
0	92.11 ± 2.85	84.81 ± 3.01	170.53 ± 7.31*	166.44 ± 5.93*
2	83.42 ± 3.71	81.53 ± 6.41	185.62 ± 4.62*	127.81 ± 5.53**
4	96.24 ± 5.38	73.14 ± 2.80♦♦	200.46 ± 10.00*	136.16 ± 9.10**
6	88.47 ± 7.81	69.51 ± 2.70♦♦	196.65 ± 9.31*	133.88 ± 3.18**

Each value is the mean ± S.E., n = 10.

* : Significantly different from corresponding normal group at each time interval, p<0.01.

♦♦ : Significantly different from normal control, p<0.05, p<0.01.

** : Significantly different from diabetic control, p<0.01.

Table 4. Plasma triglycerides (mg/100 ml) concentration in normal and diabetic rats treated with olive oil.

Time in week	Normal rats		Diabetic rats	
	Control	Olive oil	Control	Olive oil
0	80.26 ± 2.46	73.24 ± 5.06	183.26 ± 10.15*	212.09 ± 11.28*
2	75.38 ± 6.42	70.18 ± 6.35	190.65 ± 14.11*	154.76 ± 9.11**
4	76.73 ± 4.39	65.42 ± 2.16	196.42 ± 8.63*	141.50 ± 10.88**
6	78.52 ± 3.26	60.35 ± 2.80♦	210.46 ± 13.89*	111.59 ± 6.79**

Each value is the mean ± S.E., n = 10.

* : Significantly different from corresponding normal group at each time interval, p<0.01.

♦ : Significantly different from normal control, p<0.05.

** : Significantly different from diabetic control, p<0.01.

Table 5. Plasma HDL (mg/100 ml) concentration in normal and diabetic rats treated with olive oil.

Time in week	Normal rats		Diabetic rats	
	Control	Olive oil	Control	Olive oil
0	65.38 ± 2.51	70.24 ± 3.9	43.15 ± 1.17*	47.76 ± 2.67*
2	67.12 ± 3.08	76.48 ± 5.41	41.12 ± 2.13*	60.54 ± 4.19*
4	76.73 ± 4.39	75.36 ± 3.04	38.36 ± 1.19*	67.41 ± 1.94**
6	63.52 ± 2.93	81.00 ± 2.56♦	35.75 ± 3.14*	71.86 ± 4.09**

Each value is the mean ± S.E., n = 10.

* : Significantly different from corresponding normal group at each time interval, p<0.01.

♦ : Significantly different from normal control, p<0.05.

** : Significantly different from diabetic control, p<0.01.

Table 6. Plasma LDL (mg/100 ml) concentration in normal and diabetic rats.

Time in week	Normal rats		Diabetic rats	
	Control	Olive oil	Control	Olive oil
0	51.62 ± 3.82	56.09 ± 5.20	80.56 ± 6.24*	76.27 ± 3.19*
2	48.15 ± 3.21	58.14 ± 4.06	86.38 ± 4.26*	47.06 ± 4.02**
4	55.26 ± 4.10	51.36 ± 3.80	90.11 ± 5.82*	47.45 ± 5.51**
6	56.38 ± 3.93	48.26 ± 2.73♦	92.00 ± 4.88*	50.95 ± 3.24**

Each value is the mean ± S.E., n = 10.

* : Significantly different from corresponding normal group at each time interval, p<0.01.

♦ : Significantly different from normal control, p<0.05.

** : Significantly different from diabetic control, p<0.01.

3. Cholesterol concentration

Plasma cholesterol concentration was elevated significantly (p<0.01) in diabetic rats at all time intervals (Table 3). The Cholesterol level decreased significantly in normal rats given olive oil after 4 and 6 weeks (p<0.05, p<0.01). Administration of olive oil to diabetic rats led to a decrease in cholesterol level significantly (p<0.01) after 2, 4 and 6 weeks.

4. Triglycerides level

The mean levels of triglycerides (Table 4) were significantly higher (p<0.01) in diabetic rats when compared to normal ones. The treatment of normal rats with olive oil results in a significant (p<0.05) decrease of triglyceride concentration after 6 weeks. The triglyceride level was lowered significantly (p<0.01) in diabetic rats given olive oil at all time intervals as compared to diabetic control.

5. HDL level

Plasma HDL concentration decreased significantly (p<0.01) in diabetic rats as compared to normal ones

(Table 5). HDL level was significantly (p<0.05) increased in the normal rats administered olive oil after 6 weeks. Moreover, HDL level elevated significantly in diabetic rats treated with olive oil at all time intervals.

6. LDL level

Plasma LDL was affected by alloxan or olive oil (Table 6). It was observed that LDL concentration of diabetic control rats elevated significantly (p<0.01) as compared to normal control at all time intervals. On the other hand, LDL concentration of diabetic rats treated with olive oil is significantly (p<0.01) lower than that of control diabetic at any time intervals. No significant difference in LDL Concentration was observed between diabetic and normal rats given olive oil.

Discussion

It has been proved that hyperglycemia generates oxidative stress leading to the development of diabetic complications (Baynes, 1991). Peroxidation of membrane lipids associated with increased membrane rigidity, and reduced cells survival has been implicated in diabetes mellitus (Selvam and Anuradha, 1988). The observed increase in hydroperoxide level in diabetic rats could be attributed to the increase in peroxidative damage of lipids, thereby contributing to alterations in lipids and antioxidant status.

Hypercholesterolemia and hypertriglyceridemia are independent risk factors that alone or together can accelerate the development of coronary artery disease and progression of atherosclerotic lesions (Mc Kenney, 2001) Diabetes mellitus is one of the most common metabolic diseases and the derangements in lipid metabolism (Fumelli *et al.*, 1996). Increased plasma cholesterol in diabetic rats of the present experiments may be due to diminishing in clearance from blood. Plasma LDL can undergo reuptake in the liver via specific receptors and get cleared from the circulation. This increase in plasma LDL concentration may be due to defective receptors for LDL. HDL can be protective by reversing cholesterol transport, inhibiting the oxidation of LDL and by neutralizing the atherogenic effects of oxidized LDL. HDL helps to scavenge cholesterol from extra hepatic tissues (Brewer, 2004). Decreased HDL can contribute to the increased cholesterol levels. A greater increase of LDL may cause a greater decrease of HDL as

there is a reciprocal relation between the concentration of LDL and HDL. Oxidized LDL is thought to promote atherogenesis by increased lipid peroxidation (Nishigake *et al.*, 1981).

A significant increase in lipid peroxidation in diabetic rats suggests that increased generation of free radicals by hyperglycemia related to glucose auto-oxidation (Woeff and Dean, 1987). Therefore, the levels of lipid peroxides were significantly higher in diabetic rats as compared with control. After administration of olive oil, the levels of lipid peroxides declined significantly in both normal and diabetic rats. The antioxidant activity of olive oil has been reported (Aguilera *et al.*, 2003). Some findings demonstrate that olive oil phenolics are powerful antioxidants, both *in vivo* and *in vitro*, and possess other potent biological activities that could partially account for the observed healthful effect of the Mediterranean diet (Visoli *et al.*, 2002). Olive oil elevated the activities of hepatic antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase. (Ruiz-Gutierrez *et al.*, 1999).

Olive oil is highly enriched in oleic acid (unsaturated fatty acid) which results in a decrease of LDL, cholesterol and triglycerides levels of hypercholesterolemic patients (Sirtori *et al.*, 1992). These effects may be also due to sitosterol of Olive oil which affects lipids (Becker *et al.*, 1992). Feeding rabbits or humans with oleic acid rich diet reduced LDL concentration in plasma (Aviram and Eias, 1993). In the present experiments, we recorded a decrease in the plasma levels of total cholesterol, triglycerides, LDL and an increase of HDL concentration of rats given olive oil either normal or diabetic. There is evidence linking increased serum cholesterol to a higher risk for developing coronary heart disease (Glueck *et al.*, 1986). LDL is a major risk factor, whereas HDL is a protective factor for heart diseases, moreover, HDL involved in the degradation of cholesterol (Castelli *et al.*, 1986). Polyunsaturated fatty acids from olive oil have hypocholesterolemic activity (Reaven *et al.*, 1993). Oxidative modification of LDL cholesterol is thought to play an initiating role in the development of atherosclerosis (Berliner and Heinecke, 1996). Diets high in unsaturated fatty acids have been shown to reduce the susceptibility of LDL to oxidative modification (Abbey *et al.*, 1993). The correction of hyperglycemia could be related to the lowering triglyceride concentration in

olive oil supplemented diabetic rats.

In conclusion, these results suggest that there is an increased oxidative stress and concentration of lipids in plasma in diabetic rats. Olive oil shows antioxidant activity and reduced the level of plasma lipids which was elevated in diabetic control rats.

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References

- Abbey, M.; Belling, G. B.; Noakes, M.; Hirata, F.; Nestel, P. J. 1993. Oxidation of low density lipoproteins: intraindividual variability and the effect of dietary linoleate supplementation. *Am. J. Clin. Nutr.* 57: 391-398.
- Aguilera, C. M.; Mesa, M. D.; Ramirez-Tortosa, M. C.; Quiles, J. L.; Gil, A. 2003. Virgin olive and fish oils enhance the hepatic defense system in atherosclerotic rabbits. *Clinical Nutrition.* 22 (4): 379-384.
- Aviram, M.; Eias, K. 1993. Dietary olive oil reduces low-density lipoprotein uptake by macrophages and decreases the susceptibility of the lipoprotein to undergo lipid peroxidation. *Ann. Nutr. Metabol.* 37 (2): 75-84.
- Baynes, J. W. 1991. Role of oxidative stress in development of complications in diabetes. *Diabetes.* 40: 405-411.
- Becker, M.; Staab, D.; Von, B. K. 1992. Longterm treatment of severe familial hypercholesterolemia in children: effect of sitosterol and bezafibrate. *pediat.* 89 (1): 138-142.
- Berliner, J. A.; Heinecke, J. W. 1996. The role of oxidized lipoproteins in atherogenesis. *Free Rad. Biol. Med.* 20: 707-727.
- Brewer, H. B. 2004. Focus on high density lipoproteins in reducing cardiovascular risk. *Am. Heart. J.* 148: S14-S18.
- Castelli, W. P.; Garrison, R. J.; Wilson, P. W.; Abbott, R. D.; Kalousdian, S.; Kannel, W. B. 1986. Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham study. *JAMA.* 256: 2835-2838.
- De La Puerta, R.; Gutierrez, V. R.; Houtl, J. R. 1999. Inhibition of leukocyte 5-lipoxygenase by phenolics from virgin olive oil. *Biochem. pharmacol.* 57: 445-449.
- Glueck, C. J.; Gordon, D. J.; Nelson, J. J.; Davis, C. E.; Tyroler, H. A. 1986. Dietary and other correlates of changes in total and low density lipoprotein cholesterol in hypercholesterolemic men: the lipid research clinics

- coronary primary prevention trial. *Am. J. Clin. Nutr.* 44: 489-500.
- Godin, D. V.; Wohaieb, S. A.; Garnett, M. E. Goumeniouk, A. D. 1988. Antioxidant enzyme alterations in experimental and clinical diabetes. *Mol. Cell. Biochem.* 84: 223-231.
- Fumelli, P.; Romagnoli, F.; Carlino, G.; Fumelli, C.; Bomei, M. 1996. Diabetes mellitus and chronic heart failure. *Arch Gerontol. Geriatr.* 23: 277-281.
- McKenney, J. M. 2001. Pharmacotherapy of dyslipidemia. *Cardiovasc Drugs. Ther.* 15: 413-422.
- Montedoro, G. F.; Servili, M.; Baldioli, M.; Miniati, E. 1992. Simple and hydrolysable phenolic compounds in virgin olive oil. Their extraction, separation, and quantitative and semiquantitative evaluation by HPLC. *J. Aricult. Food Chem.* 40: 1571-1576.
- Nishigake, I.; Hagihara, M.; Tsunekawa, H.; Maseki, M.; Yagi, K. 1981. Lipid peroxide levels of serum lipoprotein fraction of diabetic patients. *Biol. Chem. Med.* 25: 373-378.
- Panneerselvam, S. R. and Govindasamy, S. 2004. Effect of Sodium molybdate on the status of lipids, lipid peroxidation and antioxidant systems in alloxan-induced diabetic rats. *Clin. Chem. Acta.* 345 (1-2): 93-98.
- Petroni, A.; Balsevich, M.; Salami, M.; Papini, N. Montedoro, G. F.; Galli, C. 1995. Inhibition of platelet aggregation and eicosanoid production by phenolic components of olive oil. *Thromb. Res.* 78: 151-160.
- Reaven, P.; Parthasarathy, S.; Grasse, B. J.; Miller, E.; Steinberg, D.; Witztum, J. L. 1993. Effects of oleate rich and linoleate-rich diets on the susceptibility of low density lipoprotein to oxidative modification in mildly hypercholesterolemic subjects. *J. Clin. Invest.* 91: 668-676.
- Ruiz-Gutierrez, V.; Perez-Espinosa, A.; Vazquez, C. M.; Santa-Maria, C. 1999. Effects of dietary fats (Fish, olive and high oleic acid sunflower oils) on lipid composition and antioxidant enzymes in rat liver. *Br. J. Nutr.* 82 (3): 233-241.
- Selvam, R. and Anuradha, C. V. 1988. Lipid peroxidation and antiperoxidative enzyme changes in erythrocyte in diabetes mellitus. *Indian J. Biochem. Biophys.* 25: 268-272.
- Sirtori, G. R.; Gatti, E.; Tremoli, E.; Galli, G.; Gain-Franceschi, G.; Franceschini, G. Stragliotto, E. 1992. Olive oil, Corn oil and n-3 Fatty acids differently, affect lipid lipoprotein, Platelets and superoxide Formation in type II hypercholesterolemia. *Am. J. Clin. Nutr.* 56 (1): 113-122.
- Visoli, F. and Galli, C. 1998. Olive oil phenols and their potential effects on human health. *J. Agric. Food Chem.* 46: 4292-4296.
- Visoli, F.; Poli, A.; Galli, C. 2002. Antioxidant and other biological activities of phenols from olives and olive oil. *Med. Res. Rev.* 22 (1): 65-75.
- Woeffl, S. P. and Dean, R. T. 1987. Glucose auto-oxidation and protein modification. The potential role of autooxidative glycosylation in diabetes. *Biochem. J.* 247: 243-250.

التأثير المضاد للأكسدة والحافض للدهون لزيت الزيتون في ذكور الجرذان العادية والمصابة بداء السكري

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الملخص

تعود الأعراض المرضية المتنوعة لداء البول السكري الى أسباب متعددة. اهتم هذا البحث بدراسة مستوى الهيدروبروكسيد الدهني، ومستوى الدهون في بلازما الجرذان المصابة بداء السكري معمليا بالالوكسان، ثم عولجت بزيت الزيتون لمدة ستة أسابيع. ارتفع مستوى الهيدروبروكسيد، والكليسترول، والجلسريدات الثلاثية، والبروتينات الدهنية منخفضة الكثافة (LDL)، بينما انخفضت البروتينات الدهنية عالية الكثافة (HDL) في الحيوانات المصابة بالسكري. وعند المعالجة بزيت الزيتون ظهر تحسن في مستوى الدهون وانخفاض في مستوى الهيدروبروكسيد في المجموعتين. نوقشت النتائج من خلال خصائص زيت الزيتون المضادة للأكسدة.

الكلمات الكاشفة : زيت الزيتون، البول السكري، الدهون، مضادات الأكسدة.