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PII: S0308-8146(98)00067-3

Food Chemistry, Vol. 63, No. 4, pp. 453–459, 1998
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Printed in Great Britain
0308-8146/98 $19.00 + 0.00

Hypocholesterolemic effect of dietary evening primrose (*Oenothera paradoxa*) cake extract in rats

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(Received 3 November 1997; revised version accepted 23 January 1998)

The objective of this study was to determine the effects of dietary evening primrose cake extract (E) on the lipid indices and lipid peroxidation products in growing rats fed cholesterol-free standard diet (S) or diets enriched with 1% cholesterol (C). All animals were divided into five groups of 10 and were fed during 4 weeks experimental diets: group (Gr) 1–standard diet (S); Gr 2–S diet supplemented with 1% E (S + 1%E); Gr 3–with 1% C (S + 1%C); Gr 4–S + 1%C + 0.5%E and Gr 5–S + 1%C + 1%E. Dietary E significantly decreased plasma total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) but did not change the high density lipoprotein cholesterol (HDL-C) either in rats fed cholesterol-free or enriched-in-cholesterol diets. Plasma TC were 98.7, 81.3, 144.6, 114.5 and 99.1 mg $100\,ml^{-1}$, whereas LDL-C values were 19.4, 7.6, 77.2, 43.6 and 27.6 mg $100\,ml^{-1}$ in Groups 1–5, respectively. Supplementation of diet with E significantly elevated triglyceride and phospholipid concentrations in the liver; also 1% E with C (S + 1%C + 1%E) caused significant TC accumulation in the liver and elevated malondialdehyde concentrations in plasma and erythrocytes. In conclusion, this study demonstrates, for the first time, that evening primrose cake extract (E) possesses strong hypocholesterolemic action. Its antioxidative properties, especially in animals loaded with dietary cholesterol, are less clear and need further studies. © 1998 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

The relationship between high levels of plasma lipids and the incidence of atherosclerosis and cardiovascular disease has been long recognised. The most convincing evidence is the fact that, in animal species, atherosclerosis may be experimentally induced by the use of hypercholesterolemic diets. In rats and other species administration of dietary cholesterol increases low density lipoprotein cholesterol (LDL-C) and decreases high density cholesterol (HDL-C) (Mahley and Holcombe, 1977;). It is often considered that elevated plasma cholesterol, especially LDL-C, is a predisposing factor for atherosclerosis and cardiovascular disease, frequently occurring in industrialized countries (Diaz *et al*., 1997; Goldstein and Brown, 1987; Steinberg, 1983). In contrast to that, HDL-C may exert a protective effect (Mattson and Grundy, 1985).

Free radical-mediated lipid peroxidation has been implicated in a variety of pathological processes (Ames *et al*., 1992; Aruoma *et al*., 1991; Kehrer, 1993), especially in the initiation and promotion of atherosclerosis (Henning and Chow, 1988; Henriksson *et al*., 1985; Prasad and Kalra, 1993; Steinberg *et al*., 1989; Steinberg, 1992). Increased production of superoxide anion in hypercholesterolemic vessels contributes to the atherosclerotic process (Ohara *et al*., 1993). It has been reported that hypercholesterolemic atherosclerosis is associated with an increased tissue concentration of lipid peroxidation products, malondialdehyde (MDA) and conjugated dienes. Many reports have suggested that the oxidation of plasma low density lipoproteins (LDL), induced by free radicals and other reactive oxygen substances (ROS), is an important preliminary event in pathogenesis of atherosclerosis in humans (Esterbauer *et al*., 1992; Steinberg *et al*., 1989). These studies suggested that, in human hypercholesterolemia, generation of ROS is increased.

Recent data suggest that antioxidant nutrients may play a key role in the prevention of cardiovascular disease (Diaz *et al*., 1997; Diplock, 1991, 1994). The protective effect of antioxidant nutrients may be mediated

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through inhibition of the oxidative modification of LDL-C.

Many vegetable foodstuffs, especially fruits and beverages, contain high amounts of polyphenols. Green tea tannins (Muramatsu *et al*., 1986) and grape seed tannins (Tebib *et al*., 1994*a*, 1997) have been reported to exert a hypocholesterolemic effect in cholesterol-fed rats. Yugarani *et al*. (1992) demonstrated that tannic acid decreased plasma total and LDL-C. Similar data were obtained with persimmon fruit (Kulasek *et al*., 1994).

It is well known that polyphenols act as free radical scavengers *in vitro* (Constantino *et al*., 1992; Frankel *et al*., 1995; Jovanovic *et al*., 1994; Vinson *et al*., 1995); tannins occur naturally in tea, wines and juices (Hertog *et al*., 1993) and exhibit antioxidant effects (Hertog *et al*., 1993; Miyara *et al*., 1993).

Evening primrose seeds are widely used as a source of gamma-linolenic acid, but the cake contains about 10% of polyphenolic compounds. Our *in vitro* experiments revealed that evening primrose seed cake extract has strong antioxidant properties, which even exceed butylatedhydroxy-anisole (BHA) (Bałasińska *et al*., 1997). The purpose of the present experiment was to investigate the effect of dietary evening primrose extract on lipid metabolites and peroxidation indices in blood, liver and kidney of rats fed standard diet and diet enriched with cholesterol.

MATERIALS AND METHODS

Animals and diets

Male Wistar rats, weighing about 100 g at the beginning of an experiment, were divided randomly into five dietary groups of 10. The experiment lasted 4 weeks. All groups received experimental semi-synthetic diets. Gr 1 was fed a standard diet (S). Gr 2 and 3 were fed the same diet supplemented with 1% evening primrose seed cake extract (S + 1%E) and 1% of cholesterol (S + 1%C), respectively. Gr 4 and 5 received diets with cholesterol (1%) and additionally supplemented with 0.5%E (S + 1%C + 0.5%E) and 1%E (S + 1%C + 1%E), respectively (Table 1).

All animals were kept in individual stainless steel metabolic cages at constant temperature (25°C ± 1°C) with a 12-h light:dark cycle. Water and feed were provided *ad libitum*. Food intake was recorded daily; body weight was recorded once a week. Protocol of the experiment was approved by the Animal Care Committee at Warsaw Agricultural University.

After 4 weeks, rats were sacrificed by intraperitoneal injection of pentobarbital. Blood was collected by cardiac puncture into heparin-moistened syringes, and centrifuged at 1000 ***g*** for 15 min at 4°C to separate plasma and erythrocytes. Liver and kidney were quickly removed. All samples of tissues, erythrocyte and plasma were refrigerated in liquid nitrogen and stored at -80°C until assay.

Preparation of polyphenolic extracts

Evening primrose (*Oenothera paradoxa*) cake was obtained from seeds previously deprived of oil by the cold press method and was a gift of Agropharm®, Poland. The cake was defatted with diethyl ether and then extracted with acetone:water (7:3) for 3 h at 50°C in a shaking incubator (5 l extraction fluid on 1 kg cake). The extract was filtered and acetone was evaporated in a vacuum; the residue was refrigerated and lyophilized. The extract powder (E) was used to prepare experimental diets. It contained 70.5 g polyphenols per 100 g powder. Total phenols were analyzed according to the method of Folin-Ciocalteu (Singleton and Rossi, 1965), in which phenols were calibrated against tannic acid used as a standard and the results were expressed as tannic acid.

Analytical procedures

Concentrations of total cholesterol (TC), HDL-cholesterol (HDL-C), triglycerides (TG) and phospholipids (PL) in plasma were analyzed colorimetrically using diagnostic kits (BioMerioux, France). Plasma LDL-cholesterol (LDL-C) was calculated using the following formula: LDL-C = TC−(HDL-C)−TG/5 (Tsi *et al*., 1995).

Liver and kidney total cholesterol (TC), triglycerides (TG) and phospholipids (PL), as well as erythrocyte phospholipids were determined by an enzymatic colorimetric method using a kit (BioMerioux, France). Lipid extraction was carried out according to the procedure of Folch *et al*. (1957). Briefly, about 100 mg of tissue from each rat was homogenized in 9 ml of chloroform:methanol mixture (2:1), then 3 ml of 0.9% NaCl was added, vortex-mixed and centrifuged at 1000 ***g*** for 10 min. The organic extract (OE) was removed and used for assay.

Lipid peroxidation

Lipid peroxidation was evaluated by measuring thiobarbituric acid reactive species and conjugated dienes. Thiobarbituric acid assay for the determination of malondialdehyde (MDA) levels was done by the colorimetric method (Rice-Evans *et al*., 1991). Briefly, 1 ml of organic extract (OE) was evaporated and 2.5 ml 0.05 M H_2SO_4 and 2.5 ml 1% thiobarbituric acid were added to the residue. In plasma, instead of OE, 0.5 ml of plasma was used. The mixture was vortexed, incubated for 60 min in boiling water, then extracted with n-butanol. The absorbance of the sample was determined at 532 nm against blank. The MDA concentration was calculated from a standard curve using 1,1,3,3-tetramethoxypropane as standard which gives MDA upon hydrolysis during the assay. Conjugated dienes were assayed by the method of Pryor and Castle (1984). Briefly, 0.5 ml organic extract was evaporated to dryness under nitrogen, then dissolved in cyclohexane. The absorbance of samples was measured at 234 nm. Results were calculated as per g tissue ($A_{234nm}\,g^{-1}$ tissue).

Table 1. Composition (%) of diets

Ingredient	Group				
	1 Standard (S)	2 S + 1%E	3 S + 1%C	4 S + 1%C + 0.5%E	5 S + 1%C + 1%E
Casein	20	20	20	20	20
Wheat starch	60	59	59	58.5	58
Potato starch	5	5	5	5	5
Sunflower oil	10	10	10	10	10
Mineral mix*	3.5	3.5	3.5	3.5	3.5
Vitamin mix**	1.3	1.3	1.3	1.3	1.3
Choline	0.2	0.2	0.2	0.2	0.2
Cholesterol (C)	—	—	1	1	1
Extract of evening primrose seeds oil-free cake (E)	—	1	—	0.5	1

*Mineral mix composition (g kg^{-1}): $CaHPO_4*2H_2O$, 27.89; K_2PO_4, 2.43; K_2SO_4, 2.04; NaCl, 0.92; $Na_2HPO_4*12H_2O$, 1.61; MgO, 0.75; $C_3H_4(OH)(COO)_3Fe*3H_2O$, 37.85; $Zn(CH_3COOH)_2*2H_2O$, 7.88; $MnCO_3$, 23.41; $Cu(CH_3COO)_2*H_2O$, 4.95; KJ, 0.04; $C_3H_4(OH)(COOH)_3$, 25.87.
**Vitamin mix composition (units or g kg^{-1}): vit.A, 2000 000 U; D_3, 200 000 U; E, 10 000 U; para amino benzoic acid, 10 g; inositol, 10 g; vit. PP, 4 g; pantothenic acid, 4 g; vit. B_2, 0.8 g; vit. B_1, 0.5 g; vit. B_6, 0.5 g; vit. B_{12}, 0.003 g; folic acid, 0.2 g; biotin, 0.04 g; wheat starch Q.S.P. 1000 g.

Statistical analysis

Data were evaluated using two-way analysis of variance (ANOVA) and Tuckey's test (Statgraphics). Values of $p < 0.05$ were considered to be statistically significant.

RESULTS

Growth and food consumption

Final body weight and body weight gains of rats were not affected by experimental treatments (data not shown). Also, feed efficiency, as expressed by gain/feed ratio, did not differ among groups.

Tissue

Plasma total and LDL cholesterol (TC and LDL-C) were highest in Gr 3 receiving dietary cholesterol (Table 2). Addition of evening primrose cake extract significantly decreased TC and LDL-C levels in a dose-responsive manner, not only in Gr 2 (S + 1%E) fed standard diet, but also in Gr 4 (S + 1%C + 0.5%E) and Gr 5 (S + 1%C + 1%E) receiving diet enriched with cholesterol. In Gr 5, TC level even returned to the control values (Gr 1). Plasma HDL-C was significantly decreased in rats receiving dietary cholesterol. Addition of evening primrose cake extract in Gr 4 and 5 did not completely restore HDL-C level to the value of Gr 1; however, a small rise was observed (NS). Atherogenic index, expressed by the ratio of HDL-C/(TC−HDL-C), significantly decreased in Gr 3 receiving dietary cholesterol. Addition of evening primrose cake extract significantly increased that index in Gr 2 (S + 1%E) fed diet without cholesterol. In Gr 4 and 5 addition of evening primrose abolished the suppressive effect of dietary cholesterol in a dose-responsive manner.

Supplementation of a diet with cholesterol alone evoked a significant rise in total cholesterol in the liver (Gr 3 vs 1). Addition of evening primrose cake extract increased total liver cholesterol in Gr 5 (Table 2).

Plasma triglycerides and phospholipids were not affected by dietary cholesterol. In contrast, dietary supplementation with evening primrose extract significantly increased liver TG and PL and also plasma PL in rats (Table 2).

Plasma and erythrocyte MDA concentrations were highest in rats from Gr 5. Animals fed diet supplemented with cholesterol alone (Gr 3) were characterized by higher conjugated diene levels in liver and kidney than animals fed standard diet (Gr 1); supplementation with the extract significantly lowered conjugated diene levels in kidney, but not in liver (Table 3).

DISCUSSION

Recently, much attention has been focussed on the protective biochemical function of naturally occurring antioxidants in plants and on the mechanisms of their action. Phenolic compounds, which occur widely in plants, were considered for a long time to be antioxidants (Fürst, 1996; Pratt, 1992; Pratt and Hudson, 1990). Seeds of evening primrose are rich in polyphenolic compounds; most of them are present in the fat-free residue (cake). Our previous experiment *in vitro* revealed that evening primrose cake extract and its fractions possess higher antioxidative properties than butylatedhydroxy-anisole (Bałasińska *et al.*, 1997). This study is the first attempt to demonstrate that evening primrose cake extract could affect plasma and tissue lipid parameters in rats fed

Table 2. Effect of evening primrose oil-free cake extract (E) on the plasma and liver lipid concentrations in rats fed on the standard and high cholesterol diet

Items	Group				
	1 Standard (S)	2 S + 1%E	3 S + 1%C	4 S + 1%C + 0.5%E	5 S + 1%C + 1%E
Plasma					
TC [mg 100 ml^{-1}]*	98.7 ± 2.4^{b}**	81.3 ± 1.5^{a}	144.6 ± 2.5^{d}	114.5 ± 2.4^{c}	99.1 ± 2.3^{b}
HDL-C [mg 100 ml^{-1}]	64.4 ± 2.3^{c}	59.2 ± 1.4^{bc}	50.8 ± 2.0^{a}	55.1 ± 2.2^{ab}	54.9 ± 1.9^{ab}
LDL-C [mg 100 ml^{-1}]	19.4 ± 3.1^{b}	7.6 ± 1.3^{a}	77.2 ± 2.0^{e}	43.6 ± 1.2^{d}	27.6 ± 1.4^{c}
PL [mg 100 ml^{-1}	157.4 ± 3.5^{a}	179.2 ± 3.1^{b}	174.6 ± 3.3^{ab}	162.5 ± 5.6^{ab}	176.5 ± 6.0^{b}
TG [mg 100 ml^{-1}]	75.4 ± 6.2^{a}	72.7 ± 4.3^{a}	82.5 ± 1.8^{a}	78.8 ± 4.5^{a}	82.4 ± 2.6^{a}
HDL-C/TC ratio	0.65 ± 0.03^{d}	0.73 ± 0.02^{e}	0.35 ± 0.01^{a}	0.48 ± 0.01^{b}	0.55 ± 0.01^{c}
HDL-C/(TC-HDL-C) atherogenic index	2.01 ± 0.27^{c}	2.90 ± 0.38^{d}	0.54 ± 0.03^{a}	0.93 ± 0.04^{ab}	1.25 ± 0.06^{b}
Liver					
TC [mg g^{-1} tissue]	2.5 ± 0.2^{a}	3.0 ± 0.1^{a}	22.1 ± 1.0^{b}	24.5 ± 1.2^{b}	32.2 ± 1.3^{c}
PL [mg g^{-1} tissue]	17.4 ± 0.6^{a}	23.1 ± 1.1^{b}	17.3 ± 0.6^{a}	21.9 ± 0.9^{b}	22.6 ± 0.9^{b}
TG [mg g^{-1} tissue]	5.2 ± 0.4^{a}	7.2 ± 0.2^{b}	5.1 ± 0.3^{a}	7.4 ± 0.5^{b}	7.9 ± 0.5^{b}

*Abbreviations: TC–total cholesterol; TG–triglycerides; PL–phospholipids; HDL-C–high density lipoprotein cholesterol; LDL-C–low density lipoprotein cholesterol.
**Values are means ± SEM of 10 observation per group; values in a row with different superscripts letters are significantly different ($p < 0.05$).

Table 3. Effect of evening primrose seeds oil-free cake extract (E) on malondialdehyde (MDA) and conjugated diene concentrations in plasma, liver, kidney and erythrocyte of rats fed standard and high cholesterol diet

Items	Group				
	1 Standard (S)	2 S + 1%E	3 S + 1%C	4 S + 1%C + 0.5%E	5 S + 1%C + 1%E
Plasma					
MDA [nmol ml^{-1}]*	14.6 ± 0.5^{a}**	12.9 ± 0.7^{a}	17.1 ± 0.7^{b}	16.5 ± 0.5^{b}	19.2 ± 0.7^{c}
Liver					
MDA [nmol g^{-1} tissue]	571.5 ± 33.7^{c}	438.5 ± 17.4^{ab}	378.2 ± 11.8^{a}	402.9 ± 24.9^{a}	510.1 ± 27.9^{bc}
conjugated dienes [A_{234nm} g^{-1} tissue]	3.7 ± 0.2^{a}	3.84 ± 0.4^{a}	6.4 ± 0.2^{b}	7.1 ± 0.3^{b}	6.2 ± 0.2^{b}
Kidney					
MDA [nmol g^{-1} tissue]	589.2 ± 22.3^{ab}	664.7 ± 37.6^{b}	669.4 ± 60.1^{b}	522.4 ± 24.4^{ab}	449.1 ± 36.3^{a}
conjugated dienes [A_{234nm} g^{-1} tissue]	1.5 ± 0.08^{a}	1.7 ± 0.04^{ab}	1.9 ± 0.08^{b}	1.5 ± 0.07^{a}	1.4 ± 0.05^{a}
Erythrocyte					
MDA [nmol mg^{-1} PL]	0.15 ± 0.01^{a}	0.13 ± 0.01^{a}	0.24 ± 0.01^{b}	0.15 ± 0.01^{a}	0.58 ± 0.02^{c}

*Abbreviations: MDA–malondialdehyde; PL–phospholipids.
**Values are means ± SEM of 10 observations per group; values in a row with different superscripts letters are significantly different ($p < 0.05$).

standard diet (cholesterol-free) and diet with cholesterol (1%). It is known that some polyphenolic compounds of plant origin decrease plasma cholesterol in rats fed hypocholesterolemic diet (Kulasek *et al*., 1994; Tebib *et al*., 1994*b*, 1997). Our data indicate that evening primrose extract has a profound hypocholesterolemic effect, not only in rats fed diets enriched with cholesterol, but also in animals fed standard diet without cholesterol (S vs S + 1%E). Addition of extract to a hypercholesterolemic diet for rats prevented rise in plasma cholesterol in a dose-responsive manner. Present results also show, that addition of cholesterol alone increased plasma LDL-cholesterol in rats compared with animals fed standard diet (S vs S + 1%C); dietary polyphenols (E) act conversely, namely they inhibit cholesterol-related rise in plasma LDL-cholesterol and even significantly decrease its level when animals receive standard diet (Table 2). The anti-atherogenic index [HDL-C/(TC−HDL-C)] and the HDL-C/TC ratio were significantly elevated in cholesterol/extract-treated Gr 4 and 5 compared with Gr 3 as well as in rats fed cholesterol-free diet (S vs S + 1%E) (Table 2). These effects were beneficial since it is known that there is an inverse relationship between these HDL indices and a risk of atherosclerosis and cardiovascular disease (Goldstein and Brown, 1987). Therefore, the observed rise in these indices together with decreased plasma LDL-C level may indicate that extract from evening primrose cake may play an important protective role

against atherosclerosis and cardiovascular disease in mammals.

The mechanism underlying these effects is not clear. Tebib *et al.* (1994*a*) observed increased faecal cholesterol excretion in rats receiving grape tannins, especially in polymeric form, which in turn could decrease intestinal cholesterol absorption. Elsewhere, an increase in the excretion of bile acids in faeces was noted in rats fed diet containing condensed tannins (Horigome *et al.*, 1988). Since Kuyvenhoven *et al.* (1989) demonstrated that an increased excretion of faecal bile acids would decrease body cholesterol resulting in a lower blood cholesterol, an increased excretion of bile acids may be another mechanism by which polyphenols from evening primrose cake extract prevent rise in plasma TC and LDL-C (Table 2).

Liver total cholesterol was elevated in Gr 3 receiving dietary cholesterol. Evening primrose extract did not suppress cholesterol content in the liver. In contrast, regardless of whether rats were fed standard diet or cholesterol diets, there was always a rise in liver cholesterol which turned to be significant only in Gr 3 vs Gr 5. This might be the consequences of reverse cholesterol transport, such as the movement of cholesterol from extrahepatic tissues to the liver cholesterol (Millner, 1990). In our experiment, it is difficult to explain the exact mechanism of the elevated TC content in liver due to the extract administration. Moreover, concentrations of TG and PL in Gr 2, 4 and 5 receiving evening primrose extract were also elevated. These results, that is the rise in liver TC, together with the rise in the liver TG and PL concentrations in these groups compared with Gr 1 and 3, seem to indicate that evening primrose extract, in addition to its beneficial effects, may also increase the infiltration of fat into the hepatocytes. Therefore routine administration of high amounts of extract, at least in rats, has to be taken with caution due to the risk of liver steatosis.

The measurement of MDA and conjugated dienes allowed us to assess lipid peroxidation. Plasma MDA concentration was lower in groups receiving cholesterol-free diet (Gr 1, 2). In groups fed cholesterol, addition of extract increased MDA level in Gr 5. Although addition of evening primrose extract decreased the level of LDL-C in Gr 4 and 5, this level was still significantly elevated when compared with Gr 1. Highest ratio [HDL-C/(TC−HDL-C)] observed in Group 2 was accompanied by the lowest MDA level. In Gr 3 the ratio HDL-C/(TC−HDL-C) was lowest, but MDA level was similar in Gr 4. It could be expected that plasma LDL would be less oxidized, strengthening the beneficial effect of the decreased LDL-C concentration. Ng and Hegele (1993) suggested that HDL-C particles may have an antioxidant effect and also the ability to reduce LDL uptake. In our experimental model, addition of extract from evening primrose cake, together with a high dose of cholesterol, did not show any antioxidant effect, since plasma MDA level was similar in all groups, except Gr 5 where significant rise was noted. However, a non-significant decrease of plasma MDA content (about 12%) was observed in Gr 2 compared with Gr 1. This, in turn, may indicate that the extract still possesses some slight antioxidative properties which in our experimental design were masked by a very high content of dietary cholesterol. In the kidney, MDA and conjugated diene levels were significantly reduced in cholesterol-treated rats receiving the highest amount of extract. These data suggest that kidney of rats fed evening primrose extract is less susceptible to peroxidative damage caused by a hypocholesterolemic diet; addition of 1% extract to the diet may have some protective effect on this process.

Liver response was different; the supply of evening primrose extract decreased MDA level in that organ in rats fed cholesterol-free diet, whereas in animals treated with cholesterol an opposite effect was observed, namely a rise in MDA content. MDA concentration in erythrocyte shows that addition of dietary cholesterol (Gr 3) significantly increased MDA level in erythrocytes compared with Gr 1 and 2. In groups treated with cholesterol there was significant difference between 0.5 and 1% extract added to the diet. Smaller amount of extract decreased MDA content by 38% to the values observed in erythrocytes from Gr 1. In contrast, 1% extract increased (over 3-fold) the MDA level. At the moment, there is difficulty is explaining that phenomenon (Table 3).

Absorption of polyphenols takes place in the large intestine (Manach *et al.*, 1996). Liver is a major site for the metabolism of polyphenols, this organ has the capacity to oxidize (introduction of hydroxyl groups), reduce (carbonyl group), methylate (creation of O-methyl ester) or conjugate (glucuro- or sulpho-conjugation) polyphenols. Intestinal microflora could convert dietary polyphenols or their biliary conjugated derivatives into phenolic acid. Although the phenolic compounds have similar chemical properties, their reducing capacity is not a very precise indicator of their antioxidant activity (Rice-Evans *et al.*, 1996). The action of polyphenols depends on their structure, lipophilicity, solubility and partition between the aqueous and lipid phases where these compounds might be active (result of its action on organism depends on its solubility in physiological fluids). In our experiment we did not evaluate the detailed structure of polyphenols present in the extract. We know that this extract has antioxidant activity *in vitro* where conditions are constant and most appropriate for maintaining the reaction (Bałasińska *et al.*, 1997). In our experimental model performed *in vivo*, animals were loaded with a fairly high amount of exogenous cholesterol. In such hypocholesterolemic conditions polyphenols can be transformed into antagonistic compounds which may lose their antioxidative properties. More research is needed to evaluate individual components from evening primrose extract for better understanding the interactions between the phenolic compounds and their changes due to aging.

In conclusion, present data demonstrate that extract from evening primrose cake lowers plasma cholesterol and LDL-C and elevates HDL indices, not only in rats fed standard free-cholesterol diet, but also in rats fed diets with high cholesterol content. In turn, antioxidative properties of extract, expressed by a decrease of plasma and tissue MDA and conjugated dienes, were noted to be very homologous. Present data do not explain the mechanism responsible for the decrease of plasma cholesterol and LDL-cholesterol in experimental animals. Further studies are needed for the better understanding of the composition of extract and for estimation of the optimum ratios between the type of diet, cholesterol content and the amount of added antioxidants.

REFERENCES

Ames, B.N., Shigenaga, M.K. and Hagen, T.M. (1992) Oxidants, antioxidants, and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences USA* **90**, 7915–7922.

Aruoma, O.I., Kaur, H. and Halliwell, B. (1991) Oxygen free radicals and human diseases. *Journal of the Royal Society of Health* **111**, 172–177.

Bałasińska, B., Ostaszewski, P., Troszyńska, A. and Łatosz, A. (1997) Bioactive substances in evening primrose seeds. Deutscher Lebensmittelchemikertag, 16-18 September 1997, Berlin, p. 96.

Constantino, L., Albasini, A., Rastelli, G. and Benvenuti, S. (1992) Activity of polyphenolic crude extracts as scavengers of superoxide radicals and inhibitors of xanthine oxidase. *Planta Medica* **58**, 342–344.

Diaz, M.N., Frei, B., Vita, J.A. and Keaney, J.F. (1997) Antioxidants and atherosclerotic heart disease. *New England Journal of Medicine* **337**, 408–416.

Diplock, A.T. (1991) Antioxidant nutrients and disease prevention. An overview. *American Journal of Clinical Nutrition* **53**, 189S–193S.

Diplock, A.T. (1994) Antioxidants and disease prevention. *Molecular Aspects of Medicine* **15**, 293–376.

Esterbauer, H., Gebicki, J., Puhl, H. and Jurgens, G. (1992) The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radical Biology and Medicine* **13**, 341–390.

Folch, J., Less, M. and Sloane-Stanley, G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissue. *Journal of Biological Chemistry* **226**, 497–509.

Frankel, E.N., Waterhouse, A.L. and Teissedre, P.L. (1995) Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *Journal of Agricultural Food Chemistry* **43**, 890–894.

Fürst, P. (1996) The role of antioxidants in nutritional support. *Proceedings of the Nutrition Society* **55**, 945–961.

Goldstein, J.L. and Brown, M.S. (1987) The low density lipoprotein pathway and its relation to atherosclerosis. *Annual Review of Biochemistry* **46**, 897–930.

Henning, B. and Chow, C.K. (1988) Lipid peroxidation and endothelial cell injury, implication in atherosclerosis. *Free Radical Biology and Medicine* **4**, 99–106.

Henriksson, P., Bergstrom, K. and Edhag, O. (1985) Experimental atherosclerosis and a possible generation of free radicals. *Thrombosis Research* **38**, 195–198.

Hertog, M.G.L., Feskens, E.J.M., Hollman, P.C.H., Katan, M.B. and Kromhout, D. (1993) Dietary antioxidant flavonoids and risk of coronary heart disease, the Zutphen elderly study. *Lancet* **342**, 1007–1011.

Horigome, T., Kumar, R. and Okamoto, K. (1988) Effect of condensed tannins prepared from leaves of fodder plants on digestive enzymes *in vitro* and in the intestine of rats. *British Journal of Nutrition* **60**, 275–285.

Jovanovic, S.V., Steenken, S., Tosic, M., Marjanovic, B. and Simic, M.G. (1994) Flavonoids as antioxidants. *Journal of the American Chemical Society* **116**, pp. 4846-4851.

Kehrer, J.P. (1993) Free radicals as mediators of tissue injury and disease. *Critical Reviews in Toxicology* **23**, 21–48.

Kulasek, G.W., Bartnikowsa, E., Gorinstein, S., Leontowicz, M., Zemser, M. and Morawiec, M. (1994) Persimmon fruit polyphenols in prevention of hypercholesterolemia in rats fed diet with cholesterol. In *Bioactive Substances in Food of Plant Origin. Proc. Euro Food Tox. IV*, v. 2 eds. H. Kozłowska, J. Fornal, and Z. Zdańczyk, Olsztyn, Poland, p. 438.

Kuyvenhoven, M.R., Roszowski, W.F., West, C.E., Hoongenboom, R.L.A.P., Vos, R.M.E., Beynen, A.C. and Van der Meer, R. (1989) Digestibility of casein, formaldehyde treated-casein and soya-bean protein in relation to their effects on serum cholesterol in rabbits. *British Journal of Nutrition* **62**, 331–342.

Mahley, R.W. and Holcombe, K.S. (1977) Alterations of the plasma lipoproteins and apoproteins following cholesterol feeding in the rat. *Journal of Lipid Research* **18**, 314–324.

Manach, C., Texier, O., Regerat, F., Agullo, G., Demigne, C. and Remesy, C. (1996) Dietary quercetin is recovered in rat plasma as conjugated derivatives of isorhamnetin and quercetin. *Journal of Nutrition and Biochemistry* **7**, 375–380.

Mattson, F.H. and Grundy, S.M. (1985) Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. *Journal of Lipid Research* **26**, 194–202.

Millner, N.F. (1990) Raising high density lipoprotein cholesterol: the biochemical pharmacology of reverse cholesterol transport. *Biochemical Pharmacology* **40**, 403–410.

Miyara, M., Ohtaka, H., Katayama, H., Tatsumi, Y., Miyaichi, Y. and Tomimori, T. (1993) Structure-activity relationship of flavonoids in suppressing rat liver lipid peroxidation. *Yakugaku Zasshi* **113**, 133–154.

Muramatsu, K., Fukuyo, M. and Hara, Y. (1986) Effect of green tee catechins on plasma cholesterol level in cholesterol-fed rats. *Journal of Nutrition Science and Vitaminology* **32**, 613–632.

Ng, D.S. and Hegele, R.A. (1993) High-density lipoprotein cholesterol and atherosclerosis. *Canadian Medical Association Journal* **49**, 1807–1808.

Ohara, Y., Peterson, T.E. and Harrison, D.G. (1993) Hypercholesterolemia increases endothelial superoxide anion production. *Journal of Clinical Investigations* **91**, 2546–2551.

Prasad, K. and Kalra, J. (1993) Oxygen free radicals and hypercholesterolemic atherosclerosis, effect of vitamin E. *American Heart Journal* **125**, 958–973.

Pratt, D.E. and Hudson, B.I.F. (1990) Natural antioxidants not exploited commercially. In *Food Antioxidants*, ed. B.S.F. Hudson, pp. 171-191. Elsevier Applied Science, London and New York.

Pratt, D.E. (1992) Natural antioxidants from plant material. In *Phenolic Compounds in Food and Their Effects on Health*, eds. M.-T. Huang, C.-T. Ho and C. Y. Lee pp. 54-71. American Chemical Society, New York.

Pryor, W.A. and Castle, L. (1984) Chemical methods for the detection of lipid hydroperoxides. In *Methods in Enzymology*, eds. S.R. Colovick, N.O. Kaplan pp. 293-299. Academic Press, San Diego.

Rice-Evans, C.A., Diplock, A.T. and Symons, M.C.R. (1991) *Techniques in Free Radical Research*, Elsevier, Amsterdam.

Rice-Evans, C.A., Miller, N.J. and Paganda, G. (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine* **20**, 933–956.

Singleton, V.L. and Rossi, J.A. (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* **16**, 144–158.

Steinberg, D. (1983) Lipoprotein and atherosclerosis. A look back and look ahead. *Arteriosclerosis* **3**, 283–301.

Steinberg, D., Parthasarathy, S., Carew, T.E., Khoo, J.C. and Witztum, J.L. (1989) Beyond cholesterol. modification of low density lipoprotein that increase its atherogenicity. *New England Journal of Medicine* **320**, 915–924.

Steinberg, D. (1992) Antioxidants in the prevention of human atherosclerosis. *Circulation* **85**, 2338–2344.

Tebib, K., Bitri, L., Besancon, P. and Rouanet, J.M. (1994*a*) Polymeric grape seed tannins prevent plasma cholesterol changes in high cholesterol-fed rats. *Food Chemistry* **49**, 403–406.

Tebib, K., Besancon, P. and Rouanet, J.M. (1994*b*) Dietary grape seed tannins affect lipoproteins, lipoprotein lipases and tissue lipids in rats fed hypercholesterolemic diets. *Journal of Nutrition* **124**, 2451–2457.

Tebib, K., Rouanet, J.M. and Besancon, P. (1997) Antioxidant effects of dietary polymeric grape seed tannins in tissue of rats fed a high cholesterol-vitamin E-deficient diet. *Food Chemistry* **59**, 135–141.

Tsi, D., Das, N.P. and Tan, B.K.H. (1995) Effects of aqueous celery (*Apium graveolens*) extract on lipid parameters of rats fed a high fat diet. *Planta Medica* **61**, 18–21.

Vinson, J.A., Dabbagh, Y.A., Serry, M.M. and Jang, J. (1995) Plant flavonoids, especially tea flavonols, are powerful antioxidants using an *in vitro* oxidation model for heart disease. *Journal of Agricultural Food Chemistry* **43**, 2800–2802.

Yugarani, T., Tan, B.K.H., Teh, M. and Das, N.P. (1992) Effects of polyphenolic natural products on the lipid profiles of rats fed high fat diets. *Lipids* **27**, 181–186..