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Food Supplementation with an Olive (*Olea europaea* L.) Leaf Extract Reduces Blood Pressure in Borderline Hypertensive Monozygotic Twins

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Hypertension is a harmful disease factor that develops unnoticed over time. The treatment of hypertension is aimed at an early diagnosis followed by adequate lifestyle changes rather than pharmacological treatment. The olive leaf extract EFLA®943, having antihypertensive actions in rats, was tested as a food supplement in an open study including 40 borderline hypertensive monozygotic twins. Twins of each pair were assigned to different groups receiving 500 or 1000 mg/day EFLA®943 for 8 weeks, or advice on a favourable lifestyle. Body weight, heart rate, blood pressure, glucose and lipids were measured fortnightly. Blood pressure changed significantly within pairs, depending on the dose, with mean systolic differences of ≤6 mmHg (500 mg vs control) and ≤13 mmHg (1000 vs 500 mg), and diastolic differences of ≤5 mmHg. After 8 weeks, mean blood pressure remained unchanged from baseline in controls (systolic/diastolic: 133 ± 5/77 ± 6 vs 135 ± 11/80 ± 7 mmHg) and the low-dose group (136 ± 7/77 ± 7 vs 133 ± 10/76 ± 7), but had significantly decreased for the high dose group (137 ± 10/80 ± 10 vs 126 ± 9/76 ± 6). Cholesterol levels decreased for all treatments with significant dose-dependent within-pair differences for LDL-cholesterol. None of the other parameters showed significant changes or consistent trends. Concluding, the study confirmed the antihypertensive and cholesterol-lowering action of EFLA®943 in humans.

Keywords: Olea europaea L. leaf extract; EFLA®943; blood pressure; antihypertensive effect; LDL-cholesterol.

INTRODUCTION

Hypertension is one of the most common and important disease factors imposed by the modern lifestyle, in particular by reduced physical activity and an unbalanced, lipid-rich diet. It often develops without notice and may convey or aggravate possible fatal diseases such as cardiovascular diseases (CVD) or chronic heart failure. Currently, the risk of CVD is thought to increase progressively throughout the blood pressure range, beginning at 115/75 mmHg; levels exceeding 140/90 mmHg require intervention (Appel *et al.*, 2006; European Society of Hypertension, 2003; Khan *et al.*, 2005; Whitworth, 2003). Therefore, hypertension should be thoroughly controlled and treated precociously. However, recommendations to change one's lifestyle as first-line treatment often fail in early and/or asymptomatic disease stages. On the other hand, most antihypertensive drugs such as ß-blockers, calcium blockers, ACE inhibitors or diuretics have significant side effects at common doses (Sweetman, 2002), which hardly justify their use in mild forms of hypertension. A potential alternative treatment of borderline hypertension might consist of food supplementation with beneficial ingredients from natural products and plants that are known to have ameliorating effects on blood pressure.

The olive tree (*Olea europaea* L.) and in particular its leaves have been used for the treatment of wounds, fever, diabetes, gout, atherosclerosis and hypertension since ancient times (Jänicke *et al.*, 2003). However, proof of efficacy for the treatment of hypertension with olive leaves has not been consistent. A prophylactic blood pressure lowering action of the olive leaf extract EFLA®943 has been shown in a preclinical study with rats treated with L-NAME (Khayyal *et al.*, 2002). The current study aimed at measuring and confirming this effect in humans.

MATERIAL AND METHODS

Experimental design. This was an open, controlled, parallel-group, co-twin study consisting of two parallel experiments with borderline hypertensive monozygotic twins. In both experiments, twins of each pair were randomly assigned to different treatment groups; each group consisted of 10 subjects. In one experiment, a treatment group taking one film tablet containing 500 mg of EFLA®943 daily at breakfast was compared with a control group receiving no medication, but

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advice on how hypertension may be ameliorated by an adequate lifestyle. In the other experiment, the daily dose of 500 mg was compared with that of 1000 mg (two tablets, each with a meal, one in the morning and one in the evening). The duration of both experiments was 8 weeks. Blood pressure and heart rate were measured at baseline screening, after 1 week and from week 2 onwards fortnightly until the end of the study. Body weight, blood glucose and blood lipids were also measured at baseline screening, and after 4 and 8 weeks of treatment. Adverse events were monitored and recorded throughout the study.

Study population. The study population consisted of 40 monozygotic twins aged between 18 and 60 years with an untreated suboptimal blood pressure, exceeding 120 mmHg systolic or 80 mmHg diastolic at rest. All recruited patients gave written informed consent prior to the inclusion into the study.

Investigational food supplement EFLA®943. The investigational food supplement EFLA®943 was obtained from Frutarom Switzerland Ltd. The extract was manufactured from the dried leaves of *Olea europaea* L, applying an ethanol (80% m/m) extraction procedure. After a patented filtration process (EFLA®Hyperpure), the crude extract was dried. Finally, 15% (m/m) Acaciae Gummi Ph. Eur was added as a carrier together with <0.5% (m/m) of silica colloidalis anhydrica Ph. Eur. The drug to extract ratio (DER) was 3–7:1. Characteristic components in the extract were 18–26% (m/m) oleuropein, 30–40% (m/m) polyphenols as well as verbascoside and luteolin-7-glucoside. The batch used had a DER of 5.3:1 and an oleuropein content of 20.8% (m/m). EFLA®943 was administered as film tablets containing 500 mg of extract each and excipiens pro compresso. The tablets had a final weight of 802 mg and a dimension of 19 × 8 mm.

Analytical and statistical methods. Blood pressure and heart rate were measured on each occasion by the same investigator using a device from Dinamap, Tampa, USA. Measurements were carried out on the same arm while the subject was sitting, after 5 min of rest. Blood level analyses were performed centrally by common standard analytical methods.

Mean within-pair differences of twins assigned to different groups were corrected for the difference prior to treatment and afterwards compared among time points by an analysis of variance (ANOVA) using a general linear model. Absolute values were tested between groups and for temporal changes during the experiment by an ANOVA for repeated measurements. All statistical analyses were performed using SPSS, version 12. A confidence interval (CI) of 95% ($p = 0.05$) was set for the statistical analyses.

RESULTS

The subjects represented a slightly hypertensive population. There were no significant differences of demographic data and levels of efficacy parameters among groups at baseline (Table 1). During the study period, blood pressure changed significantly between corresponding twins depending on the EFLA®943 doses. The mean systolic difference reached up to 6 mmHg between the control and the 500 mg dose and up to 13 mmHg between the 500 and 1000 mg dose group after 6 weeks of treatment (Fig. 1). Concurrently, the average diastolic difference in both experiments also reached a maximum, amounting to 5 mmHg between the control and the 500 mg dose group, as well as between the 500 mg and the 1000 mg dose group. After 8 weeks of treatment, the mean blood pressure had increased from baseline in the control group (systolic 135 ± 11 vs 133 ± 5, $p > 0.1$; diastolic 80 ± 7 vs 77 ± 6, $p > 0.1$; $n = 10$),

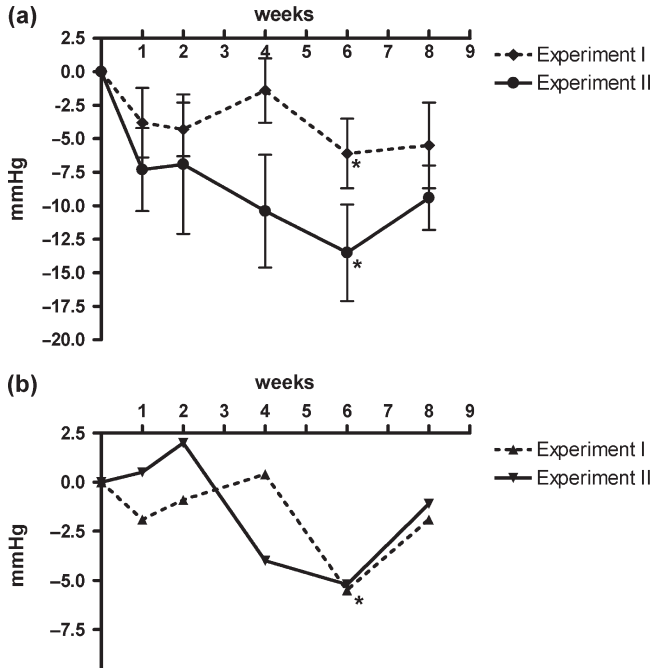

Figure 1. Mean difference ± SEM of the systolic (a) and diastolic blood pressure (b) between monozygotic twins assigned to the control and the 500 mg EFLA®943 group and those assigned to the 500 and 1000 mg EFLA®943 group. Mean values were significantly different from starting values: * $p < 0.05$.

Table 1. Demographic data (mean ± SD) of the participating monozygotic twins, no significant differences between dose groups or groups in experiment I and II

Group	Age (years)	Height (cm)	Weight (kg)	Male (*n*)	Female (*n*)	BMI (kg/m^2)
Control	38.1 ± 14.7	170 ± 6	66.2 ± 10.0	3	7	23 ± 3
500 mg	35.7 ± 14.8	170 ± 6	67.9 ± 11.0	6	14	23 ± 4
1000 mg	33.3 ± 15.4	170 ± 7	64.2 ± 8.2	3	7	22 ± 3
Total	35.7 ± 14.7	170 ± 6	66.5 ± 10.0	12	28	23 ± 3

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Table 2. Mean ± SD of blood pressure (mmHg) during the study

		Experiment I		Experiment II	
Blood pressure	Week	Control	500 mg	500 mg	1000 mg
Systolic	0	133 ± 5	135 ± 8	137 ± 7	137 ± 10
	1	132 ± 7	129 ± 10	139 ± 9	131 ± 8
	2	135 ± 10	131 ± 10	138 ± 16	130 ± 11
	4	134 ± 11	134 ± 11	141 ± 13	130 ± 8
	6	134 ± 11	129 ± 11	141 ± 14	126 ± 13
	8	135 ± 11	130 ± 11	136 ± 9	126 ± 9[a]
Diastolic	0	77 ± 6	76 ± 6	78 ± 7	80 ± 10
	1	78 ± 7	75 ± 6	79 ± 5	81 ± 9
	2	76 ± 5	74 ± 9	77 ± 7	81 ± 10
	4	77 ± 6	76 ± 5	79 ± 10	77 ± 7
	6	80 ± 6	74 ± 8	80 ± 8	76 ± 7
	8	80 ± 7	77 ± 7	75 ± 7	76 ± 6

Mean values were significantly different from starting values (screening): [a] $p < 0.01$.

decreased in the low dose group (systolic: 133 ± 10 vs 136 ± 7, $p > 0.1$; diastolic: 76 ± 7 vs 77 ± 7, $p > 0.1$; $n = 20$), and significantly decreased in the high dose group (systolic: 126 ± 9 vs 137 ± 10, $p < 0.01$; diastolic 76 ± 6 vs 80 ± 10, $p < 0.2$; $n = 10$) (Table 2). Absolute cholesterol levels showed a decrease during the course of the experiment in all treatment groups (Table 3). The differences between twins in different treatment groups were dose-dependent and significant for LDL-cholesterol (–0.50 and –0.38 mmol/L respectively, data not shown). None of the other parameters showed significant changes nor any consistent trend. No adverse events were observed throughout the trial.

DISCUSSION

This study confirmed the antihypertensive as well as the cholesterol lowering effect of the olive leaf extract EFLA®943 in rats treated with L-NAME (Khayyal *et al.*, 2002). The results also correspond to the results found with an aqueous extract from leaves of *Olea europaea* L. in patients suffering from essential hypertension (Cherif *et al.*, 1996). Moreover, the intake of extract doses of 1000 mg/day during 8 weeks was shown to be clearly superior to recommendations for lifestyle changes in subjects with borderline hypertension.

During the experiment with the 500 mg dose versus no treatment, within-pair differences were significant, although mean levels between groups and those within groups at different time points were not. This discrepancy is due to the substantially increased statistical power of the co-twin study design since determined within-pair differences allowed for a greater control of environmental factors and the detection of effects at a very sensitive level. This was confirmed by the trend towards a dose-proportionality of the effects on blood pressure and plasma LDL-cholesterol when considering the results of both experiments. However, while the effect of the 500 mg dose did not result in any significant change of mean blood pressure levels from baseline, the supplementation with 1000 mg resulted in a decrease of up to 19/10 mmHg (systolic/diastolic) within pairs and a continuous reduction of 11/5 mmHg of mean levels over 8 weeks, both suggesting clinical relevance. The increasing blood pressure levels in the control group may indicate that subjects were not compliant. However, since during the study cholesterol levels were also decreasing in this group, it may also mean that recommendations for lifestyle changes may take longer to become effective for blood pressure.

There is evidence for antihypertensive and cholesterol-lowering actions of olives and olive oil also in man (Alonso and Martinez-Gonzalez, 2004; Perona *et al.*, 2004; Psaltopoulou *et al.*, 2004), whereas for extracts

Table 3. Mean ± SD of cholesterol (mmol/L) during the study

		Experiment I		Experiment II	
Cholesterol	Time point	Control	500 mg	500 mg	1000 mg
Total	Screening	4.8 ± 1.3	4.8 ± 1.0	5.0 ± 0.9	4.7 ± 0.7
	4 weeks	4.3 ± 0.7	4.4 ± 0.9	4.3 ± 0.8	4.2 ± 0.5
	8 weeks	3.9 ± 0.7	4.1 ± 1.0	4.0 ± 0.7	3.9 ± 0.6[a]
LDL	Screening	2.6 ± 0.6	2.8 ± 0.6	2.7 ± 0.6	2.5 ± 0.5
	4 weeks	2.3 ± 0.4	2.5 ± 0.5	2.3 ± 0.6	2.2 ± 0.4
	8 weeks	2.0 ± 0.4	2.3 ± 0.7	2.3 ± 0.6	2.1 ± 0.4[a]
HDL	Screening	1.4 ± 0.4	1.3 ± 0.4	1.4 ± 0.2	1.4 ± 0.4
	4 weeks	1.4 ± 0.5	1.3 ± 0.4	1.3 ± 0.3	1.4 ± 0.3
	8 weeks	1.3 ± 0.4	1.2 ± 0.4	1.3 ± 0.3	1.3 ± 0.3

Mean values were significantly different from starting values (screening): [a] $p < 0.01$.

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from olive leaves those actions, to our knowledge, have only been reported once (Cherif *et al.*, 1996). However, both actions are well-studied in rats (Bennani-Kabchi *et al.*, 2000; Bennani-Kabchi *et al.*, 1999; De Pasquale *et al.*, 1991; Khayyal *et al.*, 2002) for which they were hypothesized to result from a combination of different effects from several active compounds (Khayyal *et al.*, 2002) of olives or olive leaves.

Oleuropein, a major component of both olives and olive leaves, was shown to be responsible for the vasodilatory effect of a decoction of leaves on isolated rat aorta preparations, but there was at least one other active principle which had either vasodilatory activity itself or potentiated that of oleuropein (Zarzuelo *et al.*, 1991). Oleuropein was also shown to enhance nitric oxide production (Visioli *et al.*, 1998b), whereas a direct calcium antagonistic activity was excluded. However, after bioassay directed fractionation, 3,4-dihydroxy-phenyl-ethanol, a degradation product of oleuropein, was shown to have such potential (Rauwald *et al.*, 1994). Other ß-glucosidase-produced metabolites of oleuropein and oleacin have been shown to inhibit the angiotensin converting enzyme (ACE) (Hansen *et al.*, 1996). A certain role in the pathogenesis of hypertension is also ascribed to oxidative stress (de Champlain *et al.*, 2004; Escobales and Crespo, 2005; Pollock, 2005; Touyz, 2004). Based on the ability of oleuropein and polyphenolic compounds to scavenge free radicals and reactive oxygen species (ROS), extracts from olive leaves have high antioxidative activity (Benavente-Garca *et al.*, 2000; Visioli *et al.*, 1998a), which specifically for EFLA®943 was shown to exceed even that of vitamin E (unpublished data). These antioxidative properties may not only contribute to the antihypertensive action, but were also suggested to be responsible for the hypocholesterolemic effect, referring to decreases of LDL and VLDL cholesterol (Bennani-Kabchi *et al.*, 2000).

In conclusion, the study confirmed the antihypertensive and cholesterol-lowering actions of the olive leaf extract EFLA®943 in humans. For the 1000 mg dose these actions were substantial in subjects with borderline hypertension whereas, for the 500 mg dose, they were only detectable using the co-twin approach. However, a dose-finding was not the aim of the current study as the combined pharmacological actions will need to be investigated further.

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