Phytomedicine 18 (2011) 251–258

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

Olive (*Olea europaea*) leaf extract effective in patients with stage-1 hypertension: Comparison with Captopril

Endang Susalit[a,1], Nafrialdi Agus[b,2], Imam Effendi[a,1], Raymond R. Tjandrawinata[c,*], Dwi Nofiarny[c,3], Tania Perrinjaquet-Moccetti[d,4], Marian Verbruggen[d,4]

[a] *Nephrology & Hypertension Division, Department of Internal Medicine, Faculty of Medicine, University of Indonesia/Dr. Cipto Mangunkusumo National General Hospital, Jl. Diponegoro 71, Jakarta 10430, Indonesia*
[b] *Pharmacology and Therapeutics Department, Faculty of Medicine, University of Indonesia/Dr. Cipto Mangunkusumo National General Hospital, Jl. Salemba 6, Central Jakarta, Indonesia*
[c] *Dexa Medica Group, Indonesia, Titan Center, 3rd Floor, Jl. Boulevard Bintaro, Block B7/B1, No. 5, Bintaro Jaya Sector 7, Tangerang 15224, Indonesia*
[d] *Frutarom Switzerland Ltd., Rütiwisstrasse 7, CH-8820 Wädenswil, Switzerland*

ARTICLE INFO

Keywords:
Olive leaf
Olea europaea
Oleuropein
Captopril
Hypertension
Stage-1
Blood pressure
Double-blind randomized study

ABSTRACT

A double-blind, randomized, parallel and active-controlled clinical study was conducted to evaluate the anti-hypertensive effect as well as the tolerability of Olive leaf extract in comparison with Captopril in patients with stage-1 hypertension. Additionally, this study also investigated the hypolipidemic effects of Olive leaf extract in such patients. It consisted of a run-in period of 4 weeks continued subsequently by an 8-week treatment period. Olive (*Olea europaea* L.) leaf extract (EFLA®943) was given orally at the dose of 500 mg twice daily in a flat-dose manner throughout the 8 weeks. Captopril was given at the dosage regimen of 12.5 mg twice daily at start. After 2 weeks, if necessary, the dose of Captopril would be titrated to 25 mg twice daily, based on subject's response to treatment. The primary efficacy endpoint was reduction in systolic blood pressure (SBP) from baseline to week-8 of treatment. The secondary efficacy endpoints were SBP as well as diastolic blood pressure (DBP) changes at every time-point evaluation and lipid profile improvement. Evaluation of BP was performed every week for 8 weeks of treatment; while of lipid profile at a 4-week interval. Mean SBP at baseline was 149.3 ± 5.58 mm Hg in Olive group and 148.4 ± 5.56 mm Hg in Captopril group; and mean DBPs were 93.9 ± 4.51 and 93.8 ± 4.88 mm Hg, respectively. After 8 weeks of treatment, both groups experienced a significant reduction of SBP as well as DBP from baseline; while such reductions were not significantly different between groups. Means of SBP reduction from baseline to the end of study were −11.5 ± 8.5 and −13.7 ± 7.6 mm Hg in Olive and Captopril groups, respectively; and those of DBP were −4.8 ± 5.5 and −6.4 ± 5.2 mm Hg, respectively. A significant reduction of triglyceride level was observed in Olive group, but not in Captopril group. In conclusion, Olive (*Olea europaea*) leaf extract, at the dosage regimen of 500 mg twice daily, was similarly effective in lowering systolic and diastolic blood pressures in subjects with stage-1 hypertension as Captopril, given at its effective dose of 12.5–25 mg twice daily.

Introduction

Hypertension affects approximately 50 million individuals in the United States and approximately 1 billion individuals worldwide. As the population ages, the prevalence of hypertension will increase even further unless broad and effective preventive measures are implemented (Chobanian et al. 2003). Excellent clinical trial outcome data prove that lowering BP with several classes of currently available anti-hypertensive drugs, including angiotensin converting enzyme (ACE) inhibitors, angitoensin-receptor blockers (ARBs), α-blockers, calcium channel blockers (CCBs) and thiazide-type diuretics, will all reduce the complications of hypertension (Chobanian et al. 2003). Nevertheless, most patients with hypertension will require two or more anti-hypertensive medications to achieve their BP goals (<140/90 mm Hg or <130/80 mm Hg for those with diabetes or chronic kidney disease) (Black et al. 2001; Cushman et al. 2002), which, on the other hand, also means the increment of risks of adverse drug reaction and medication costs.

* Corresponding author. Tel.: +62 21 7454 111; fax: +62 21 7453 111.
E-mail addresses: raymond@dexa-medica.com, liana.wijaya@dexa-medica.com (R.R. Tjandrawinata).
[1] Tel.: +62 21 314 9208; fax: +62 21 315 2278.
[2] Tel: +62 21 319 30481; fax: +62 21 392 0947.
[3] Tel.: +62 21 7454 111; fax: +62 21 7453 111.
[4] Tel.: +41 44 7826464; fax: +41 44 7826466.

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doi:10.1016/j.phymed.2010.08.016

One considerable alternative to bridge the efficacy and therapeutic costs is – using potential herbal medicines.

The leaves of the Olive tree (*Olea europaea* L.) have been used since ancient times to combat high blood pressure, atherosclerosis and diabetes and for other medicinal purposes (Jänicke et al. 2003). The anti-hypertensive and cholesterol-lowering actions of Olive leaves are well-documented (Cherif et al. 1996; Bennani-Kabchi et al. 1999, 2000; De Pasquale et al. 1991; Khayyal et al. 2002). Olive leaf contains the active substances oleuropein (a polyphenolic iridoid glycoside) (Panizzi et al. 1960), oleacein (Somova et al. 2003) and oleanolic acid (Hansen et al. 1996). EFLA®943, a stable Olive leaf extract which is standardized to oleuropein, has been preclinically studied for its safety and anti-hypertensive effects (Khayyal et al. 2002). In a preliminary clinical study carried out in 20 monozygotic adult twin pairs with mild hypertension in Germany, EFLA®943 treatment at a dose of 500 or 1000 mg daily for 8 weeks demonstrated a significant reduction of subjects' SBP and DBP, lower than those resulted in control (no treatment) group. At a dose of 1000 mg daily, the extract was clearly superior to recommendations for lifestyle changes in reducing mean blood pressure levels from baseline. The study also showed that at both doses the extract provided a significant reduction of LDL-cholesterol level (Perrinjaquet-Moccetti et al. 2008).

Based on the positive results observed in the preclinical and human studies, the current clinical trial was designed to primarily confirm the anti-hypertensive effect of the Olive leaf extract EFLA®943 in comparison with Captopril as one of the standard therapy for hypertension in patients with stage-1 hypertension. Secondary objectives of the study were to investigate the hypolipidemic effects of Olive leaf extract and to evaluate safety and tolerability of Olive leaf extract in patients with stage-1 hypertension. It is widely known that dietary changes play an important role in managing blood pressure. In highly-motivated subjects, dietary changes are effective to lower and control blood pressure, and serve as initial treatment before drug therapy in uncomplicated stage-1 hypertension (Appel et al. 1997, 2009; He and MacGregor 2004; Mulrow et al. 2008). In the light of that fact, added by the previous evidence that anti-hypertensive effect of the Olive leaf extract had demonstrated its superiority over the recommended life-style changes (Perrinjaquet-Moccetti et al. 2008), a two-arm randomized controlled trial involving non-responsive-to-diet-alone subjects with an active anti-hypertensive agent as the control was regarded appropriate to answer the study objectives.

Materials and methods

The protocol, the consent form, and the patient information sheet were reviewed and approved by independent Ethics Committee (University of Indonesia, Jakarta, Indonesia), prior to trial initiation.

The trial was performed in accordance with the Declaration of Helsinki and Good Clinical Practice. Written informed consent from every study subjects was obtained prior to any trial-related activities and the investigator retained the consent forms.

Study design

This was a randomized, double-blind, active-controlled clinical study consisting of a 4-week single-blind placebo (is diet-alone) run-in period and followed by a 8-week double-blind treatment period with: active control drug (Captopril) or Olive leaf extract. Run-in period was of important necessity in order to screen out those who responded well to diet-alone.

Subjects eligible for the study were those with stage-1 hypertension, as defined by clinic SBP of 140–159 mm Hg, with DBP of either <90 mm Hg (who were classified as isolated systolic hypertension, ISH) or in between 90 and 99 mm Hg, at screening and after the run-in period visit, either naïve or being under treatment with any anti-hypertensive medication, aged between 25 and 60 years old at screening. Those subjects which had been under anti-hypertensive medication were asked to stop their medication during participation in the study and were enrolled only upon agreement.

The exclusion criteria were: history of secondary hypertension, such as hyperaldosteronism, pheochromocytoma, renal artery stenosis, cushing syndrome; presence of target-organ damage (renal failure, congestive heart failure, myocardial infarction or cerebrovascular accident 6 months preceeding to the study), second- or third-degree heart block, valvular heart disease; diabetic subjects; hepatic dysfunction; any disease state which judged by the investigator could interfere with trial participation or trial evaluation; known or suspected allergy to the trial product or the related products; and participation in any other clinical studies within 30 days prior to screening. Pregnant and breast-feeding female subjects were not allowed to participate.

Patients were withdrawn from the trial at the discretion of the investigator if judged non-compliant with trial procedures or due to safety concerns. A patient was also withdrawn if: (1) after the 4 weeks of run-in period their SBP was <140 or >159 mm Hg or DBP was >99 mm Hg, or (2) at any time during the study, an increase in SBP of >15 mm Hg versus baseline value (after the run-in period) or an SBP of >170 mm Hg or DBP of >110 mm Hg was observed.

Eligible patients were enrolled in the study and instructed to follow a dietary advice during their participation in the study. After a 4-week run-in period, those who were still eligible according to the inclusion and exclusion criteria would receive a randomization number allocating them to receive either active control or study drugs and be scheduled to come for weekly follow-up visits. At each visit, all patients should bring the diary and remaining trial medication (including used and unused bottle-packs) given at previous visit. Throughout the study, the enrolled subjects were instructed to maintain a low fat, low sodium diet (low in saturated and total fat, low cholesterol, NaCl intake <2.4 g per day). Each subject was given a diary to record their daily diets and any deviation to the advised one. They were also given an information page simply mentioning what kinds of diet should be maintained and what to be limited during the study.

In this study, weekly visits were also considered as the best way to minimize the odds of incompliance as well as loss-to-follow-up events. Two staffs of the investigator team were dedicatedly assigned to tightly and routinely monitor the compliance of the enrolled subjects with the study protocol. Such a monitoring on subjects' compliance was performed by reminding subjects of the diet advice and checking whether or not they had taken their study medication properly, both by phone and by visiting them right at their homes in between their study-visit schedules.

Efficacy and safety endpoints

The primary efficacy endpoint was reduction of clinical SBP from baseline to week-8. The secondary endpoints were (1) reduction of clinical SBP and DBP from baseline to each post-treatment visit, (2) improvement of lipid profile (changes in fasting plasma LDL-, HDL-, total-cholesterol and triglyceride levels) from baseline to week-4 and week-8.

Safety endpoints were clinically significant changes of laboratory parameters, such as routine haematology, serum electrolyte (sodium, potassium, chloride), liver function (serum alanine-aminotransferase, ALT and serum aspartate-aminotransferase, AST levels), renal function (serum creatinine level) and adverse events either reported by subjects or observed by the investigator.

Study medication and regimen

Study medication during the treatment period was: (1) Olive leaf extract – each film coated caplet (Livitens®, Dexa Medica) contains 500 mg extract Benolea® (EFLA®943) and excipiens pro compresso; or (2) Captopril (Dexacap®, Dexa Medica) tablets at 12.5 mg. The extract, obtained from Frutarom Switzerland Ltd., 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. Characteristic component in the extract is 16–24% (m/m) oleuropein, the batch used had a content of 19.9% (m/m).

Study medications were given in a double-blind double-dummy fashion. Dummies of each medication contained the same ingredients as the respective active preparations but without the active substances. Any medications (systemic administration), including herbals, other than trial drugs that were considered to interfere with investigational drug's activity, such as anti-hypertensives, lipid-altering agents, and corticosteroids, were not allowed to be taken during the study. Anticoagulants or anti-platelets might still be concomitantly used in caution.

For the 4-week-run-in period (week −4 to week 0), dummy of Olive leaf extract was administered once a caplet daily. During the 8 weeks of treatment period, Olive leaf extract was given in a flat-dose fashion of 500 mg twice daily. Captopril was administered at an initial dose of 12.5 mg twice daily for the first 2 weeks. In both groups, patient's response to treatment was assessed after 2 weeks of treatment, at which if patient's SBP was already <140 mm Hg or there was a minimal decrease of 5 mm Hg in SBP from baseline, the patient would remain at the initial dose, otherwise either dummy Captopril (to non-responders in the Olive group) or Captopril (to non-responders in the Captopril group) would be titrated to 25 mg (or two capsules) twice daily for the rest duration of the study.

In case of serious adverse events, the trial drug was stopped immediately and the patient treated with appropriate therapy/medication.

Statistical analysis

The hypothesis of interest for the primary efficacy variable was that mean reduction of clinic SBP by Olive leaf extract was at least equivalent to that of Captopril.

Sample size estimation was calculated based on independent-*t*-test, using the primary efficacy endpoint. A study reported a clinic SBP change of 9 mm Hg by a one-year Captopril treatment (Materson et al. 1993). Using a standard deviation (SD) of 11 mm Hg (Materson et al. 1993), a significance level of 0.05, and a statistical power of 80% to detect a maximum clinically insignificant difference of 5 mm Hg between Olive and Captopril groups, a total of 144 subjects (72 subjects per arm) resulted to be required to complete the trial.

Independent-*t*-test assuming normal distribution of the data was used in the analyses of the primary efficacy endpoint between groups. Changes in all other secondary parameters were also tabulated and statistically analyzed between groups by independent-*t*-test. Pre- and post-treatment (baseline and end of treatment) analysis of both primary and secondary endpoints was performed within group using paired-*t*-test.

Throughout the analysis, variables might have been log-transformed in order to meet the underlying distributional assumptions of the statistical models; or the corresponding non-parametric test was used. All statistical tests were at 5% significance level.

Included in the safety analysis were all patients who took at least one dose of trial product. Safety assessment was based on laboratory examinations (including haematology parameters, electrolytes, liver and renal functions) and reported/observed adverse events. All laboratory parameters before and after treatment were tabulated by group and analyzed individually by paired-*t*-test or by Wilcoxon if the data were not normally distributed. Adverse events were summarized by system organ class and WHO ARD preferred term. The tabulated summary consisted of the number of subjects with event, the percentage of exposed with event and the number of events. Additionally, a patient-by-patient listing was provided, as well as a listing of all individual adverse events together with the corresponding attributes such as frequency, severity and relation to study drug.

SPSS version 14.0 was used for the analyses.

Results

Patient disposition

From October 2007 to August 2008, 232 patients referred to Nephrology & Hypertension Division, Department of Internal Medicine, of Medicine, University of Indonesia, were enrolled in the study. Of them, 162 (69.8%) subjects completed the study, 16 (6.9%) dropped out from the study due to various reasons as listed in Fig. 1, and 54 (23.3%) had no available post-treatment data. Among those subjects that completed the study, 14 (6.0%) were incompliant with respect to study medication (consumption of study medication <80%), resulting in 148 (63.8%) patients evaluable for per-protocol efficacy analysis. Among those subjects without available post-treatment data, one was actually a screening failure; the remaining had their SBP decreased to the normal range (43 subjects), or risen to stage-II hypertension (10 subjects) after the first 4-week run-in period, and were thus withdrawn from the study. The overall patient disposition is summarized in Fig. 1.

Demographic and baseline characteristics

As shown in Table 1, both groups had comparable demographic and baseline characteristics. Mean age of participants in the Olive and Captopril group was 51.5 ± 5.8 and 49.7 ± 6.8 years, respectively, both groups were predominantly composed of female subjects (85.4% and 87.6%, respectively). With regards to BMI category, only 2–3% of subjects in each group had normal body mass index, while underweight, overweight and obese subjects were evenly distributed between both groups. LDL- and total-cholesterol levels at baseline were somewhat higher in the Olive group than in the Captopril group, the proportion of those subjects with high LDL-cholesterol levels (>130 mg/dl) was also higher in the Olive group. However, compared were the differences from baseline to study end, instead of the end of study's results, therefore the slightly different baseline values would not much affect the evaluation.

Efficacy evaluation

The efficacy results are tabulated in Tables 2–4. All efficacy endpoints were analyzed based on PP populations including 148 patients.

Effects on blood pressures

At the end of study, there were 28 (36.8%) subjects in Captopril group whose dose regimen needed to be titrated to 25 mg twice daily. While in Olive group, dummy Captopril was titrated

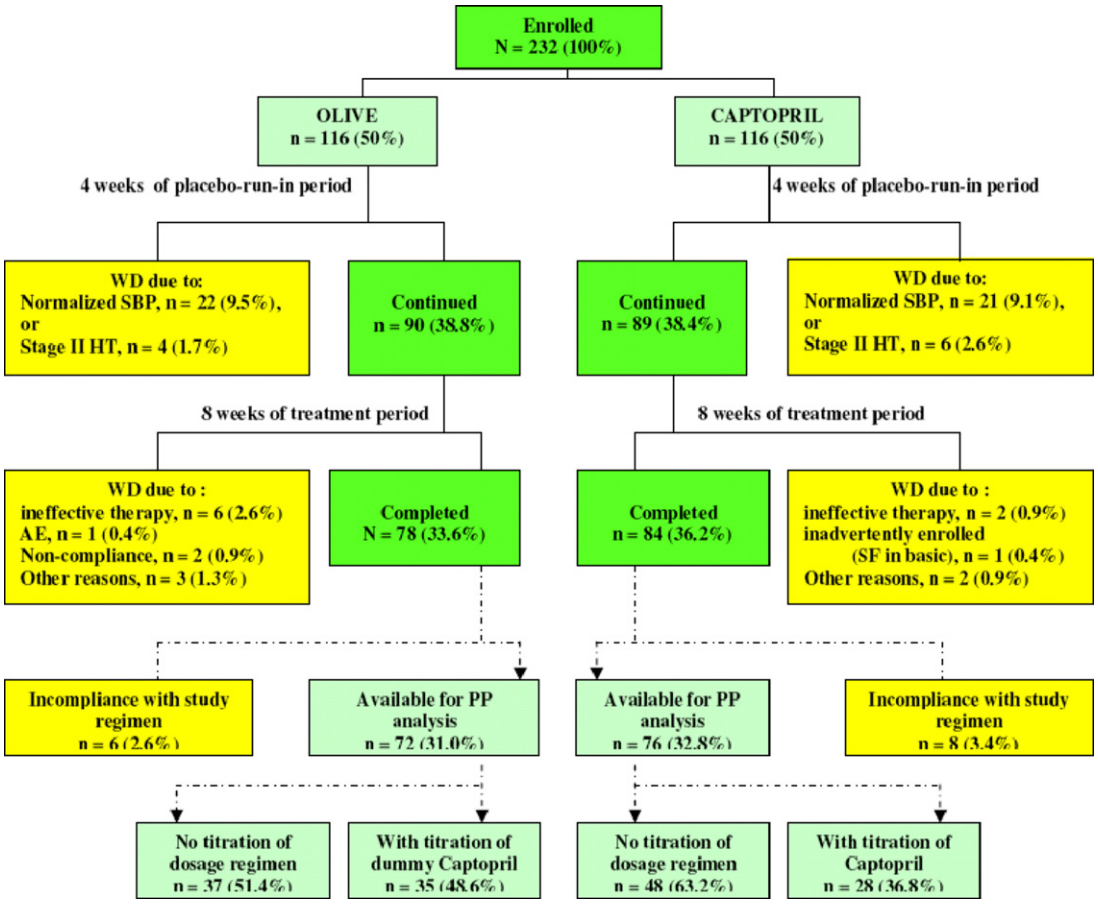

Fig. 1. Patient disposition. ITT, intent-to-treat; SBP, systolic blood pressure; SF, screened failure; WD, withdrawn.

up to two tablets twice daily in 35 (48.6%) subjects (Fig. 1). The Olive leaf extract demonstrated a comparable blood pressure lowering effect (-11.5 ± 8.6 and -4.8 ± 5.5 mm Hg for SBP and DBP, respectively) to that shown by Captopril (-13.7 ± 7.6 and -6.4 ± 5.2 mm Hg for SBP and DBP, respectively), see Table 2. The difference between groups in terms of reduction of SBP and DBP were found statistically and clinically insignificant ($p > 0.05$).

Effects on lipid profile

Administration of Olive leaf extract also resulted in the improvement of the lipid profile as shown by a significant reduction of total-cholesterol and triglyceride levels, and a slight reduction of LDL-cholesterol from baseline; however, no effect on HDL-cholesterol level was observed. Such beneficial effects on cholesterol levels were not found in the Captopril group. LDL reduction from baseline to week-8 of treatment was significantly greater ($p = 0.032$) in the Olive (-3.89 ± 19.40 mg/dl) than in the Captopril group (2.14 ± 14.20 mg/dl) (Table 2). Furthermore, the significant reduction of triglyceride levels due to the Olive leaf extract amounted to -11.90 ± 46.17 mg/dl, compared to almost no changes found with Captopril treatment (-1.26 ± 43.31 mg/dl). In the subgroup of subjects with high baseline triglyceride level (>200 mg/dl), the effect of the Olive leaf extract was even more remarkable (Fig. 2): the triglyceride level diminished up to 23.2% (-53.13 ± 58.71 mg/dl) from baseline, compared to 13.9% reduction (-38.44 ± 53.53 mg/dl) in the Captopril group. The difference between groups in the subset analysis was not statistically significant as the statistical power of that subset was too low to detect such a difference.

Safety evaluation

Before and after treatment data of laboratory safety parameters are shown in Table 4.

At the end of treatment, the following laboratory parameters were found statistically different from baseline: ALT, haematocrit,

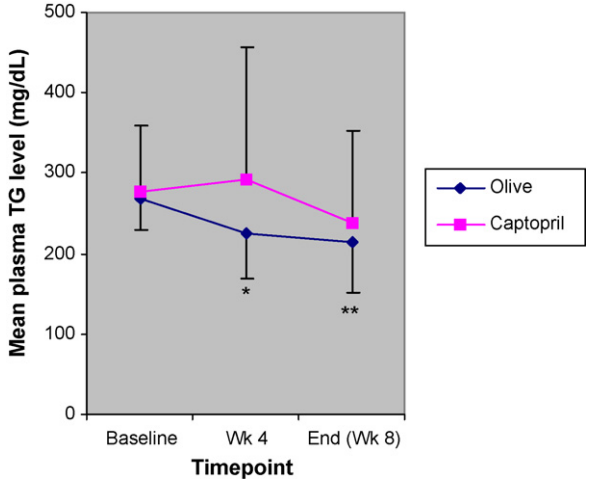

Fig. 2. Plasma Triglyceride levels throughout the study in the subgroup with baseline triglyceride level of > 200 mg/dl. The number of subjects in Olive and Captopril groups were 15 and 9, respectively; *, significantly different ($p < 0.05$) with baseline value; **, significantly different ($p < 0.01$) with baseline value; TG, triglyceride; Wk, week; End, end of study (after 8 weeks of treatment). No significant differences between groups, particularly due to the low statistical power.

Table 1
Demography and baseline characteristics.

Characteristic	PP ($N = 148$)		
	Olive ($n = 72$)	Captopril ($n = 76$)	p
Age (y) – mean (SD)	51.5 (5.8)	49.7 (6.8)	N/A
Gender			
Male – n (%)	12 (16.7)	10 (13.2)	N/A
Female – n (%)	60 (83.3)	66 (86.2)	
BMI (kg/m^2) – mean (SD)	26.2 (4.15)	26.7 (4.40)	N/A
BMI category			
Underweight – n (%)	27 (37.5)	21 (27.6)	
Normal – n (%)	2 (2.8)	2 (2.6)	N/A
Overweight – n (%)	18 (25.0)	21 (27.6)	
Obese – n (%)	25 (34.7)	32 (42.1)	
Cigarette smoking			
None – n (%)	57 (73.6)	53 (69.7)	
Abstinent–n (%)	8 (11.1)	5 (6.6)	N/A
Smoker – n (%)	11 (15.3)	18 (23.7)	
Alcoholism			
None – n (%)	65 (90.3)	68 (89.5)	N/A
Abstinent – n (%)	5 (6.9)	6 (7.9)	
Alcoholic – n (%)	2 (2.8)	2 (2.6)	
At screening			
Systolic BP (mm Hg) – mean (SD)	149.5 (5.93)	147.9 (6.17)	0.194
Diastolic BP (mm Hg) – mean (SD)	93.9 (4.46)	93.5 (4.90)	0.825
Duration of Hypertension (years) – mean (SD)	2.55 (5.77)	2.98 (5.59)	0.732
FPG (mg/dl) – mean (SD)	90.8 (9.85)	91.6 (10.12)	0.627
LDL-C (mg/dl) – mean (SD)	141.1 (31.50)	128.9 (29.20)	0.015
HDL-C (mg/dl) – mean (SD)	50.5 (11.81)	51.0 (11.01)	0.807
TC (mg/dl) – mean (SD)	206.2 (33.6)	190.1 (31.16)	0.003*
TG (mg/dl) – mean (SD)	126.2 (51.75)	113.2 (59.80)	0.053
At baseline (after a 4-week run-in period)			
Systolic BP (mm Hg) – mean (SD)	145.0 (5.00)	144.7 (4.45)	0.850
Diastolic BP (mm Hg) – mean (SD)	91.3 (5.14)	89.8 (6.60)	0.286
LDL-C (mg/dl) – mean (SD)	136.4 (32.18)	122.3 (25.73)	0.004*
Normal LDL-C– n (%)	34 (47.2)	49 (65.5)	0.026*
High LDL-C (>130 mg/dl) – n (%)	38 (52.8)	27 (35.5)	
HDL-C (mg/dl) – mean (SD)	48.3 (11.30)	47.9 (9.13)	0.819
TC (mg/dl) – mean (SD)	202.2 (34.6)	183.6 (27.47)	<0.001*
TG (mg/dl) – mean (SD)	140.3 (68.15)	119.5 (63.04)	0.055
Normal TG – n (%)	62 (86.1)	70 (92.1)	0.182
High TG (>200 mg/dl) – n (%)	10 (13.9)	6 (7.9)	

PP, per-protocol analysis; BP, blood pressure; FPG, fasting plasma glucose; AST, serum glutamic–oxaloacetic transferase; ALT, serum glutamic–piruvic transferase; LDL-C, low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol; TC, total-cholesterol; TG, triglyceride.
* Significantly different (at $\alpha = 0.05$) between groups.

platelet and potassium levels in the Olive group, ALT, serum creatinine, haemoglobin, haematocrit, red blood count and white blood count in the Captopril group. However, such changes were not clinically relevant since the absolute values of each parameter at the end of treatment were all still within their respective normal range and there was only a slight shift from their baseline values (Table 4). Other laboratory safety parameters showed no difference at the end of treatment as compared to baseline.

A total of 1057 adverse events were reported by 168 (94.4%) study subjects, 83 subjects (49.4%) belonged to the Olive group and 85 (50.6%) to the Captopril group. The majority of adverse events were tolerably mild (99.8%) and comparable between groups. The most common adverse events which contributed to more than 5% of the total events observed during the study were coughing (4.6% in Olive and 7.0% in Captopril group) and vertigo (5.9% in Olive and 6.3% in Captopril group). Less frequently, muscle discomfort, headache, fatigue, malaise, myalgia and muscle cramp were reported and comparable between groups, constituting less than 5% of the total events. One event (in Olive group) fulfilled the criteria of serious adverse event. This subject suffered from severe anaemia following her persistent menorrhagia. The subject was hospitalized until the anaemia was recovered and her condition stabilized. Based on her history of abortion and curettage, it can be concluded that there was no relationship between this AE and the study medication. Only coughing was probably related to Captopril, as this adverse drug reaction is widely known to occur following Captopril intake, especially in elderly patients. Vertigo, muscle discomfort and headache were judged to be possibly related to both Olive leaf extract and Captopril. All these adverse events had resolved at the end of the study. At the end of study, all adverse events experienced by the subjects had either recovered or stabilized.

Discussion

The anti-hypertensive and cholesterol-lowering actions of the Olive leaf extract (EFLA®943) were previously demonstrated in a clinical study carried out in 20 monozygotic adult twin pairs with mild hypertension (systolic BP of 120–160 mm Hg and diastolic BP of 80–95 mm Hg) in Germany (Perrinjaquet-Moccetti et al. 2008). Consistent with the German Twin study's result, in the current study we also found a beneficial effect of the extract in subjects with stage-1 hypertension (with baseline systolic blood pressure higher than 140 but lower than 160 mm Hg). In both the Olive and the Captopril groups, there was a significant reduction of systolic as well as diastolic blood pressure after 8 weeks of treatment as compared to baseline (Table 3). In subjects with stage-1 hypertension, Olive leaf extract with a daily dose of 2× 500 mg demonstrated a blood pressure lowering activity, both on systolic and on diastolic, which was proven to be effectively comparable to, that exerted by

Table 2
Efficacy endpoints in both groups.

	Variables	Olive (n = 72)Mean (SD)	Captopril (n = 76)Mean (SD)	p	95% Confidence interval
Reduction of SBP from baseline, after x week of treatment (mm Hg)					
	1	−3.4 (8.1)	−6.2 (8.1)	0.038*	0.157 to 5.443
	2	−5.8 (9.6)	−8.5 (9.0)	0.082	−0.340 to 5.682
	3	−7.4 (8.8)	−8.5 (10.2)	0.493	−2.022 to 4.174
	4	−9.3 (8.8)	−11.0 (9.2)	0.270	−1.282 to 4.548
	5	−8.8 (8.7)	−10.8 (10.1)	0.205	−1.093 to 5.048
	6	−9.2 (9.4)	−12.5 (8.9)	0.029*	0.350 to 6.277
	7	−9.4 (8.5)	−11.9 (9.1)	0.085	−0.355 to 5.380
	8	−11.5 (8.6)	−13.7 (7.7)	0.098	−0.413 to 4.861
Reduction of DBP from baseline, after x week of treatment (mm Hg)					
	1	0.1 (5.7)	−1.1 (5.5)	0.198	−0.629 to 3.012
	2	−1.1 (5.8)	−1.9 (4.6)	0.329	−0.859 to 2.545
	3	−1.5 (5.6)	−2.7 (5.4)	0.182	−0.574 to 3.003
	4	−2.1 (6.3)	−3.4 (8.1)	0.071	−0.137 to 3.304
	5	−2.9 (5.1)	−3.7 (4.7)	0.344	−0.828 to 2.359
	6	−3.0 (6.1)	−4.4 (4.7)	0.134	−0.422 to 3.112
	7	−2.9 (5.8)	−4.3 (5.5)	0.134	−0.437 to 3.247
	8	−4.8 (5.5)	−6.4 (5.2)	0.065	−0.101 to 3.384
Change of lipid profile from baseline, after x week of treatment (mg/dL)					
LDL-C	4	−1.8 (19.6)	1.7 (13.8)	0.214	−9.052 to 2.045
	8	−3.9 (19.4)	2.1 (14.2)	0.032*	−11.536 to −0.531
HDL-C	4	0.0 (4.9)	−0.5 (5.0)	0.548	−1.113 to 2.088
	8	0.1 (5.7)	−0.9 (5.4)	0.264	−0.775 to 2.810
TC	4	−4.1 (19.8)	−1.1 (15.6)	0.311	−8.734 to 2.802
	8	−5.8 (22.2)	0.5 (17.4)	0.058	−12.690 to 0.217
TG	4	−10.0 (37.5)	−4.0 (31.4)	0.292	−17.215 to 5.219
	8	−11.9 (46.2)	−1.3 (43.3)	0.150	−25.176 to 3.897

SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol; TC, total-cholesterol; TG, triglyceride.
* Significantly different at $\alpha = 0.05$.

Captopril given at 12.5 or 25 mg (the dose was adjusted based on subject's response) twice daily (Table 2).

The mechanism of action by which Olive leaf extract exerts its anti-hypertensive effects is continuously being studied. The anti-hypertensive effects of active constituents in Olive leaf extract, such as oleuropein and oleacein, might be associated with inhibiting ACE: these components could be cleaved by β-glucosidase to derivatives with high ACE inhibitor activity (Hansen et al. 1996). Oleuropein has been recognized as one of the responsible components of a decoction of leaves having vasodilatory effect on isolated rat aorta preparations (Zarzuelo et al. 1991). A degradation product of oleuropein, 3,4-dihydroxy-phenyl-ethanol, was shown to have a direct calcium antagonistic activity (Rauwald et al. 1994). In a latest preclinical study using the isolated rabbit's heart and rat's cardiomyoctes, it was reported that Olive (*Olea europaea*) leaf extract suppressed the L-type calcium channel directly and reversibly (Scheffler et al. 2008). Therefore, the anti-hypertensive activity of the Olive leaf extract lies probably in its content of oleuropein acting synergistically with other active substances to exert both ACE inhibitory and calcium channel blocking activities. However, it still needs more studies to conclusively confirm the anti-hypertensive mechanism of the Olive leaf extract as this study was not designed to answer such a question.

In addition to its beneficial effects on systolic and diastolic blood pressures, Olive leaf extract also demonstrated its valuable effect in improving the lipid profile of stage-1 hypertensive subjects. At the study dosage regimen (500 mg twice daily for 8 weeks) the extract was found to significantly reduce total-cholesterol as well as triglyceride levels of the study subjects, by 2.8% and 7.8%, respectively (Table 3). There was also a borderline significant reduction in LDL-cholesterol (2.9%) observed in Olive group (Table 2). The result is in line with that of the German Twin study which demonstrated a significant reduction of LDL-cholesterol by the treatment of Olive leaf extract (Perrinjaquet-Moccetti et al. 2008). Such a favourable effect on cholesterol was not found with Captopril treatment. In a subset group of subjects with baseline triglyceride higher than the optimum level, i.e. >200 mg/dl (Fig. 2), the reduction in Olive group was even more remarkable (23.2%). Although the difference between groups was not statistically significant, the pre-post analysis undoubtedly showed the triglyceride lowering effect in the Olive group (Fig. 2). This finding indicates that the higher the baseline level of plasma triglyceride, the greater the reduction would be. Such reduction was even only slightly below that exerted by fenofibrate, a potent anti-triglyceride agent (Keating and Croom 2007).

The dual effect of Olive leaf extract in both reducing blood pressure and improving lipid profile is advantageous since lower levels

Table 3
Baseline and end of study values of blood pressure, total-cholesterol and triglyceride levels.

Variables	BaselineMean (SD)	EndMean (SD)	p
Olive group (n = 72)			
SBP (mm Hg)	145.0 (5.0)	133.5 (10.4)	<0.001**
DBP (mm Hg)	91.3 (5.1)	86.6 (6.9)	<0.001**
TC (mg/dl)	202.2 (34.6)	196.4 (32.0)	0.033*
TG (mg/dl)	140.3 (68.2)	128.4 (63.8)	0.032*
Captopril group (n = 76)			
SBP (mm Hg)	144.7 (4.5)	130.9 (9.3)	<0.001**
DBP (mm Hg)	89.9 (6.6)	83.4 (7.1)	<0.001**
TC (mg/dl)	183.6 (27.5)	184.1 (30.2)	0.808
TG (mg/dl)	119.5 (63.0)	118.2 (67.7)	0.800

SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total-cholesterol; TG, triglyceride; V, visit; End, end of study (after 8 weeks of treatment).
* Significantly different at $\alpha = 0.05$.
** Significantly different at $\alpha = 0.01$.

Table 4
Before–after treatment values of the laboratory safety variables.

Safety parameters	Olive group ($n = 89$)		Captopril group ($n = 89$)	
	Mean (SD)	p	Mean (SD)	p
AST level at baseline (U/L)	21.3 (5.9)		19.4 (5.8)	
AST level at end of treatment (U/L)	20.5 (5.5)	0.092	18.6 (5.0)	0.165
ALT level at baseline (U/L)	20.0 (11.0)		18.5 (9.6)	
ALT level at end of treatment (U/L)	18.2 (9.4)	0.016*	17.1 (9.5)	0.035*
Cr at baseline (mg/dl)	0.81 (0.68)		0.70 (0.16)	
Cr at end of treatment (mg/dl)	0.76 (0.16)	0.511	0.74 (0.16)	<0.001**
Hb at baseline (g/dl)	13.5 (1.4)		13.3 (1.3)	
Hb at end of treatment (g/dl)	13.3 (1.4)	0.003**	12.9 (1.3)	<0.001**
Ht at baseline (%)	40.0 (3.7)	0.001**	39.5 (3.4)	
Ht at end of treatment (%)	39.1 (3.8)		38.5 (3.4)	<0.001**
WBC at baseline ($10^3/\mu l$)	8.3 (1.8)		8.3 (1.9)	
WBC at end of treatment ($10^3/\mu l$)	8.1 (1.6)	0.107	8.0 (1.8)	0.023*
RBC at baseline ($10^3/\mu l$)	4.7 (0.4)		4.7 (0.4)	
RBC at end of treatment ($10^3/\mu l$)	4.7 (0.4)	0.110	4.6 (0.4)	0.006**
Pt at baseline ($10^3/\mu l$)	341.2 (82.2)		348.7 (73.9)	
Pt at end of treatment ($10^3/\mu l$)	324.6 (77.6)	0.001**	342.6 (79.5)	0.185
Na^+ at baseline (mmol/l)	141.3 (1.9)		141.2 (1.8)	
Na^+ at end of treatment (mmol/l)	141.5 (2.0)	0.313	141.4 (2.1)	0.386
K^+ at baseline (mmol/l)	4.1 (0.4)		4.0 (0.4)	
K^+ at end of treatment (mmol/l)	4.0 (0.4)	0.043*	4.0 (0.4)	0.351
Cl^- at baseline (mmol/l)	103.7 (1.8)		103.5 (1.9)	
Cl^- at end of treatment (mmol/l)	103.3 (1.9)	0.072	103.6 (2.1)	0.516

ALT, alanine-aminotransferase; AST, aspartate-aminotransferase; Cr, creatinine; Hb, haemoglobin; Ht, haematocrit; WBC, white blood count; RBC, red blood count; Pt, platelet; Na^+, sodium ion; K^+, potassium ion; Cl^-, chloride ion; CI, confidence interval of the difference.

* Significantly different at $\alpha = 0.05$.

** Significantly different at $\alpha = 0.01$.

are a target for reducing the risk for cardiovascular diseases. In the subgroup analysis, such triglyceride-reduction effect was also found with Captopril treatment albeit with lower magnitude than that of Olive leaf (Fig. 2). Former clinical studies with Captopril in hyperlipidemic hypertensive subjects reported rather inconsistent effects on lipid profile. Some indicated a benefit (Costa et al. 1988; Ferrara et al. 1993; Scemama et al. 1995), while some others showed neither advantageous nor deleterious effect (Catalano et al. 1992; Lacourcière et al. 1990; Lind et al. 1994; Schulz et al. 2000), consistent with the results of the current study.

Based on the laboratory safety evaluation, we observed that administration of Olive leaf extract to stage-1 hypertensive subjects did not affect liver and renal functions. Neither did it affect haematological parameters and electrolyte balance of study participants. Even though some of the laboratory safety parameters were statistically different from baseline values, all of them remained within the normal range at the end of the study period, and thus such changes were not clinically relevant. The evaluation of all safety parameters and occurrence of adverse events showed that Olive leaf extract was safe and tolerable in patients with stage-1 hypertension.

Conclusions

Olive (*Olea europaea* L.) leaf extract at the dosage regimen of 500 mg twice daily (1000 mg daily) effectively lowered systolic and diastolic blood pressures in subjects with stage-1 hypertension. The anti-hypertensive activity of the extract was comparable to that of Captopril, given at its effective dose of 12.5–25 mg twice daily. The study also demonstrated the safety and tolerability of the extract, administered orally at a dose of 500 mg twice daily. Additionally, the beneficial effects of the extract on lipid profile, particularly in reducing plasma LDL-, total-cholesterol and triglyceride levels were strongly indicated by this trial. Further studies, involving particularly subjects with dyslipidemia, are needed to confirm the extract's effect on lipid profile.

Acknowledgements

This study was supported by PT Dexa Medica and Frutarom Switzerland Ltd. We deeply thank all subjects participated in the study. The assistance of Liana W. Susanto, MBiomed (PT Dexa Medica) in the statistical analysis as well as preparation of the draft manuscript is gratefully acknowledged. We also highly appreciate Dr. Elysabeth, Dr. Melita, Dr. Ratih Indriani Hapsari, Dr. Meta Ardiana, Dr. Kurnia Pujiastuti for their support and assistance in the study conduct.

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