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Research Article

Effects of Olive Leaf Extract on Metabolic Response, Liver and Kidney Functions and Inflammatory Biomarkers in Hypertensive Patients

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Abstract

Background and Objective: Hypertension is a long-term medical condition in which the blood pressure is gradually elevated. In this project, the effects of olive leaf extract (OLE) were evaluated on metabolic response, liver and kidney functions and also biomarkers of inflammation in hypertensive patients. **Materials and Methods:** In this randomized double-blind placebo controlled clinical trial, 60 hypertensive patients, aged 30-60 years old had participated. Patients were randomly assigned into two groups to receive either OLE or placebo tablets for 12 weeks. At the beginning and end of the intervention, metabolic parameters and biomarkers of liver, kidney and inflammation were measured in sera of the participants using available laboratory methods. **Results:** Compared with the placebo, changes in parameters associated with glucose metabolism were not statistically significant ($p > 0.05$). The OLE tablets did not have significant effect on liver enzymes, total protein, albumin, urea and creatinine ($p > 0.05$), but significantly decreased interleukin-6, interleukin-8 and tumor necrosis factor alpha as inflammatory biomarkers ($p < 0.05$) in OLE group compared to the placebo group. **Conclusion:** The results concluded that inflammation as a major cause of hypertension was significantly decreased in patients using OLE tablets.

Key words: Olive leaf extract, essential hypertension, tumor necrosis factor-alpha, hypertensive patients, cardiovascular disease, interleukin, blood pressure

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

High blood pressure is one of the most common human diseases all over the world which is considered to be a major contributor to vascular disease and mortality¹. Essential hypertension is one of the main endocrine and metabolic complaints². Correlation between high level of insulin related metabolic parameters and intensified risk of hypertension in human have been documented³. It has also been documented that variations in liver enzymes such as lipase, transferases and other parameters like hepatic insulin resistance are linked to hypertension incidences⁴⁻⁶. They confirmed higher serum uric acid is likely to be superior to insulin resistance and peripheral insulin resistance has imperative effect in the progress of hypertension than hepatic insulin resistance does^{7,8}. Researchers have reported the relationship between inflammation and blood pressure and documented that some cytokines changed in hypertensive patients⁹. Currently, drugs used for the treatment of hypertensive patients include: Diuretics, beta-blockers, calcium-channel blockers and angiotensin-converting enzyme inhibitors¹⁰. Reducing mortality and morbidity due to hypertension is the ultimate goal of treating this disease, however, due to some problems such as; drug costs, contradictory effects and complications, almost half of the treated patients do not reach this goal¹¹. Today there is a keen interest in the use of herbal medicines for the treatment of diseases. These compounds seem to be a better choice for anti-inflammatory properties than synthetic agents¹². Olive (*Olea europaea*) leaf extract comprise higher quantities of polyphenols especially oleuropein. Cardio-protective, anti-oxidant and anti-inflammatory properties of OLE were documented in many studies^{13,14}. It was observed that treatment with 400 mg kg⁻¹ b.wt., OLE every day for 8 weeks to streptozotocin induced diabetic rats, glucose and insulin significantly were decreased and increased, respectively, but no significant changes were shown in serum total protein and albumin amount¹⁵. Optimistic effects of oleuropein as a major component of OLE on glucose metabolism have been documented by Abunab *et al.*¹⁶ and Fujiwara *et al.*¹⁷. Due to the rising occurrence of hypertension, multifactorial study intended at aborting and remedy is one of the universal investigate priorities. The studies regarding the olive leaf extract were limited. Therefore this study was aimed to determine the effect of olive leaf extract on glucose metabolism related parameters, liver and kidney functions and also inflammatory cytokines among patients with essential hypertension.

MATERIALS AND METHODS

Trial design and subjects: This study project was conducted from December, 2017-November, 2018 in Qazvin, central region of Iran. The study protocol was approved by the ethics committee of Qazvin University of Medical Sciences (IR.QUMS.REC.1396.240) and registered in the Iranian website for registration of clinical trials (<http://www.irct.ir>: IRCT20170430033730N5). This study was randomized double-blind placebo controlled clinical trial that was performed on 60 patients with hypertension, diagnosed based on the Seventh Report of the Joint National Committee (JNC-7)¹⁸. Before start of the study, the informed consent forms were obtained from all participants. Using computer-generated random numbers, participants were randomly allocated into two groups to take 250 mg OLE tablets (n = 30) and placebo (n = 30) twice daily for 12 weeks (Fig. 1) based on a previous study in hypertensive patients¹⁹. Hypertensive patients who had complications such as: Diabetes, kidney disease and hypo and/or hyperthyroidism were excluded from this study. About 10 mL fasting blood samples were collected from each person at baseline and after the 12 week intervention at Booali-Sina hospital laboratory affiliated to QUMS, sera were separated using centrifuge and were frozen at -80 °C until to test time²⁰.

Placebo and olive leaf extract (Olivin[®] tablet): Placebo and Olive leaf extract (Olivin[®] tablet) were designed as tablets in Medicinal Plants Research Center of Barij and manufactured by Barij Essence Pharmaceutical Company, Kashan, Iran which approved by Food and Drug Administration (IRC:1228148347). Each tablet contained 250 mg of standard extract of olive (*Olea europaea*) leaf that standardized based on the presence of at least 0.62 mg of luteolin per tablet. The extract also contains 16% oleuropein based on the British Pharmacopeia monograph and so other active flavonoids. Very briefly, to make the tablet, filler materials including microcrystalline cellulose, Ac-Di-Sol disintegrant and magnesium stearate as lubricant were used. Ultimately, in order to protect against moisture and oxygen, the base of the HPMC overlay was used for coating.

Assessment of baseline characteristics of the participants:

Body weight was determined with a digital balance (Seca, Hamburg, Germany) at the onset of the study in the cardiology. The BMI was determined as weight in kg divided by height in meters squared. Blood pressure was measured by a trained nurse using sphygmomanometer and information of medications was collected through examination and interviews with the patient²¹.

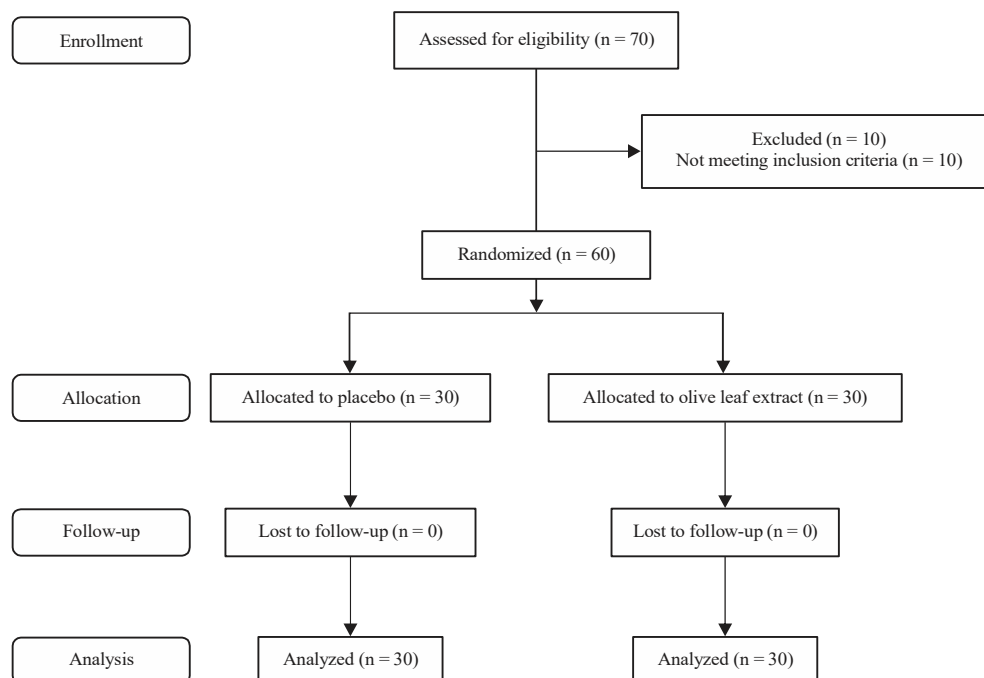


Fig. 1: Summary of patients flow diagram

Metabolic response evaluation: Fasting glucose (DIALAB kit, Vienna Austria) and insulin (DiaMetra, Milano kit, Italy) were measured by using BT-1500 auto analyzer (Biotechnica, Italy) and automated ELISA Reader (Epoch, BioTek, USA), respectively. The homeostatic model of assessment for insulin resistance (HOMA-IR). The homeostatic model of assessment for pancreatic beta-cell function (HOMA-B) and quantitative insulin sensitivity check index (QUICKI) were calculated according to suggested formulas. HOMA-IR was computed as follows:

$$\text{HOMA-IR} = \frac{\text{Fasting insulin } (\mu \text{ IU mL}^{-1}) \times \text{Fasting glucose } (\text{mmol mL}^{-1})}{22.5}$$

The HOMA-B was calculated using the following formula²²:

$$\text{HOMA-B} = \frac{20 \times \text{fasting insulin } (\mu \text{ IU mL}^{-1})}{\text{Fasting glucose } (\text{mmol mL}^{-1}) - 3.5}$$

The QUICKI was derived using the inverse of the sum of the logarithms of the fasting insulin and fasting glucose²³.

Liver and kidney functions tests: Serum transaminase enzymes AST, ALT (UV, mod. IFCC), alkaline phosphatase ALP

(colorimetric, mod. IFCC), total protein (Biuret, colorimetric), albumin (dye binding, colorimetric), uric acid (enzymatic, colorimetric), urea (enzymatic, colorimetric), uric acid (enzymatic, colorimetric) and creatinine (colorimetric, Jaffe) were measured using diagnostic kits of DIALAB company (DIALAB, Vienna Austria) by BT-1500 auto analyzer (Biotechnica, Italy).

Determining biomarkers of inflammation: Biomarkers of inflammation including IL-6, IL-8, TNF-alpha and omentin were measured by commercially available kits (Bioassay Technology Laboratory, Shanghai, China) using automated ELISA Reader (Epoch, BioTek, USA) with inter and intra-assay coefficient variances (CVs) lower than 7%.

Statistical analysis: The Kolmogorov-Smirnov test was applied to evaluate normal distribution of the variables in the study. To compare within-group (before and after treatment) and between group differences, Paired-samples and Independent t-tests were used, respectively. Results were presented as the Mean±SD and statistically determined significant at $p \leq 0.05$. Statistical analyses were done using the Statistical Package for Social Science version 23 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

There was high level of compliance in this study so more than 90% of tablets were taken by the patients. No adverse reactions were reported following consumption of OLE tablets in hypertensive patients. The baseline characteristics of the participants have been listed in Table 1. No significant differences were found between the anthropometric parameters and kind of medications of the subjects ($p>0.05$) (Table 1).

Metabolic response following OLE usage: Compared to placebo, there were no significant changes in the metabolic

responses including serum fasting blood sugar, insulin, HOMA-IR, HOMA-B and QUICKI in OLE group after 12 week intervention ($p>0.05$) (Table 2).

Effects of OLE tablets on liver and kidney functions:

In order to evaluate the effect of the tablets on liver and kidney functions, liver enzymes, total protein, albumin, uric acid, urea and creatinine were measured in serum of the subjects. As shown in Table 3, the changes in these parameters did not have a significant difference between the two groups ($p>0.05$) (Table 3).

Table 1: Baseline characteristics of the participants

Variables	Placebo group (n = 30)	OLE group (n = 30)	p ²
Sex (M/F)	13/17	14/16	0.799
Age (Year)	55.6±8.8	53.8±8.0	0.359
Duration of disease (Year)	5.1±3.2	4.3±2.6	0.273
Height (cm)	160.8±9.9	161.7±9.1	0.727
Weight (kg)	70.7±9.1	73.4±8.6	0.248
BMI (kg m ⁻²)	27.5±4.5	28.3±4.3	0.482
Systolic blood pressure (mm Hg)	140.4±5.4	142.4±6.1	0.185
Diastolic blood pressure (mm Hg)	89.7±6.5	91.4±5.1	0.258
Smoking [n (%)]	3 (10)	3 (10)	0.799
ACEI/ARB drugs [n (%)]	18 (60)	20 (66.6)	0.599
β-blocker drugs [n (%)]	14 (46.7)	13 (43.3)	0.799
Calcium channel blocker [n (%)]	15 (53.3)	16 (53.3)	0.599
Mixed [n (%)]	7 (23.3)	6 (20)	0.759

All values are Means±SD, ACEI: Angiotensin converting enzymes inhibitors, ARB: Aldosterone receptor blocker, OLE: Olive leaf extract. There were no significant differences between the variables among two studied groups ($p\text{-value}>0.05$), ²p-values represent independent samples student's t-test

Table 2: Metabolic response at study baseline and after 12 week intervention in hypertensive patients that received either OLE or placebo tablets

Table 2. Metabolic response at study baseline and after 12-week intervention in hypertensive patients that received either OLE or placebo tablets									
Variables	Placebo group (n = 30)				OLE group (n = 30)				p [‡]
	Baseline end of trial changes p [†]				Baseline end of trial changes p [†]				
FBS (mg dL ⁻¹)	94.60±11.6	95.10±9.5	0.53±10.8	0.788	90.10±11.1	91.90±9.8	1.90±12.4	0.407	0.650
Insulin (μU mL ⁻¹)	7.30±2.3	7.10±1.8	-0.13±2.6	0.788	7.70±2.1	8.10±2.0	0.48±3.1	0.405	0.412
HOMA-IR	1.70±0.63	1.68±0.50	-0.02±0.7	0.863	1.69±0.48	1.84±0.47	0.15±0.66	0.218	0.330
HOMA-B	105.20±80.2	91.40±53.4	-14.20±67.1	0.257	138.20±118.2	129.20±105.4	-8.90±143.3	0.736	0.857
QUICKI	0.37±0.02	0.36±0.02	-0.001±0.024	0.972	0.36±0.02	0.35±0.02	-0.005±0.022	0.252	0.470

All values are Means±SD, HOMA-IR: Homeostatic model of assessment for insulin resistance, HOMA-B: Homeostatic model of assessment for pancreatic beta-cell, QUICKI: Quantitative insulin sensitivity check index, FBS: Fasting blood sugar, OLE: Olive leaf extract, ¹Obtained from paired-samples t-tests, ²Represents independent samples student's t-test

Table 3: Biomarkers of liver and kidney functions at baseline and after the 12 week intervention in subjects with hypertension that received either OLE or placebo tablets

Variables	Placebo group (n = 30)				OLE group (n = 30)				p [‡]
	Baseline end of trial changes p [†]				Baseline end of trial changes p [†]				
AST (U L ⁻¹)	19.70±5.5	19.50±3.9	-0.20±4.1	0.793	19.3±4.4	19.7±5.4	0.37±5.9	0.737	0.669
ALT (U L ⁻¹)	16.20±3.1	16.70±4.7	0.43±4.8	0.628	16.2±4.9	16.9±5.1	0.73±5.3	0.456	0.820
ALP (U L ⁻¹)	110.30±11.5	110.40±14.9	0.13±22.1	0.974	116.4±16.3	110.4±14.9	-5.93±19.9	0.114	0.270
TP (g dL ⁻¹)	6.70±0.69	6.50±0.66	-0.19±0.84	0.214	6.6±0.76	6.3±0.71	-0.26±0.94	0.146	0.783
Alb (g dL ⁻¹)	0.36±0.40	0.35±0.50	-0.04±0.50	0.670	3.4±0.33	3.5±0.31	0.09±0.47	0.153	0.283
UA (mg dL ⁻¹)	5.60±1.5	5.70±1.2	0.08±1.5	0.776	5.8±1.2	5.4±1.3	-0.37±1.4	0.166	0.245
BUN (mg dL ⁻¹)	15.00±3.8	15.20±3.0	+0.07±2.9	0.901	16.7±3.7	16.2±4.0	-0.50±3.8	0.475	0.518
Cr (mg dL ⁻¹)	0.80±0.13	0.80±0.17	0.03±0.14	0.246	0.9±0.15	1.0±0.76	0.12±0.75	0.377	0.158

All values are Means±SD, AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: Alkaline Phosphatase, TP: Total Protein, UA: Uric acid, Alb: Albumin, BUN: Blood urea nitrogen, Cr: Creatinine, OLE: Olive leaf extract, ¹Obtained from paired-samples t-tests; ²Represents independent samples student's t-test

Table 4: Biomarkers of inflammation at baseline and after the 12 week intervention in subjects with hypertension that received either OLE or placebo tablets

Table 4. Biomarkers of inflammation at baseline and after the 12 week intervention in subjects with hypertension that received either OLE or placebo tablets									
Variables	Placebo group (n = 30)					OLE group (n = 30)			
	Baseline end of trial changes p [†]					Baseline end of trial changes p [†]			
									p [‡]
IL-6 (ng L ⁻¹)	77.5±12.7	78.4±13.2	0.93±11.5	0.662	75.64±11.2	69.7±9.8	-5.9±13.4	0.022	0.038*
IL- 8 (ng L ⁻¹)	143.4±13.8	142.2±10.8	0.04±12.8	0.632	139.60±16.9	131.4±11.7	-8.2±17.5	0.016	0.043*
TNF- α (ng L ⁻¹)	74.2±8.9	73.2±7.1	-1.10±12.1	0.632	75.60±8.9	67.1±7.4	-8.5±10.9	<0.001	0.015*
Omentin (ng L ⁻¹)	69.9±12.8	73.2±11.5	3.30±14.8	0.239	67.90±15.5	66.4±14.6	-1.4±20.5	0.710	0.317

*Indicated significant difference, All values are Mean±SD, OLE tablets led to significant decrease in inflammatory markers except omentin. IL-6: Interleukin-6, IL-8: Interleukin-8, TNF-a: Tumor necrosis factor-alpha, OLE: Olive leaf extract. ¹Obtained from paired-samples t-tests, ²Represents independent samples Student's t test

Effects of OLE tablets on biomarkers of inflammation:

Changes of inflammatory biomarkers following OLE tablets were significant when compared with placebo group. The OLE tablets compared to placebo, significantly decreased serum IL-6 (p-value: 0.038), IL-8 (p-value: 0.043), TNF-a (p-value: 0.015), but had no significant effect on omentin level (p = 0.317) (Table 4).

DISCUSSION

Present study assessed the effects of OLE tablets on metabolic response, liver and kidney functions and also biomarkers of inflammations in patients with essential hypertension. Compared to placebo group, taking OLE tablets had significant improvement on IL-6, IL-8 and TNF-a levels but did not have any statistically significant effect on other studied biomarkers. Anti-inflammatory and potential role of OLE in colonic samples from ulcerative colitis patients, human placenta tissue and polymorphonuclear cells cultures have been reported by Larussa *et al.*²⁴, Kaneko *et al.*²⁵ and Qabaha *et al.*²⁶. It has been described that anti-inflammatory effect of oleuropein result from its effect through reduction of NF-kB signaling pathways and decreasing IL-6 and TNF-alpha cytokines²⁷. Although the results of those studies were in agreement with the present study, but contrary to the present study, those studies were *in vitro* experiments and lacked *in vivo* comparisons. Thus, further studies especially randomized placebo-controlled trials are needed to confirm the potential health benefits of OLE in humans. The effect of OLE containing 100 mg oleuropein was evaluated on incidence of upper respiratory illness in 32 students who play sport. The results showed OLE supplementation over a season did not significantly reduce the illness incidence, but decreased duration of the illness and potentially helping them return to play²⁸. Another clinical trial that performed on only 29 male volunteers indicated OLE consumption may result in health benefits through influencing the expression of inflammatory and metabolic genes²⁹. In a randomized, controlled, crossover trial carried out by Lockyer *et al.*³⁰, it has been shown that intake of 136 mg oleuropein and 6 mg

hydroxytyrosol per day for 6 weeks had no significant effects on glucose, insulin, HOMA-IR, QUICKI and also cytokines such as; IL-6 and TNF-a. While another study examined the same compound with different doses on 46 overweight men for 12 weeks and documented that this supplementation improved QUICKI and HOMA-B, but had no effect on liver biomarkers, IL-8 and TNF-alpha³¹. These clinical studies had similarities and dissimilarities with the present study. The most important distinction between the present study and the previous publications may be related to the research design, kind of disease, nature of the extract, duration of the extract usage, patient's compliance and finally the inclusion and exclusion criteria. This study had some limitations. Thus evaluation of gene expressions related to metabolism, inflammations and oxidative stress after intervention in hypertensive patients will be the part of forthcoming studies.

CONCLUSION

Overall, OLE tablets improved the IL-6, IL-8 and TNF-α levels as compare to the placebo group without causing any diverse effect on other studied biomarkers. So, this clinical trial indicated that OLE intake after 12 weeks resulted in significant improvement of inflammation among patients with essential hypertension.

SIGNIFICANCE STATEMENT

Most of the previous studies were not clinical trials or examined only one or two effective ingredients from the extract while this trial is one of the most unique studies of its kind which investigate the effect of whole extract of olive leaf on metabolic parameters, liver and kidney functions and also inflammatory markers in patients with essential hypertension. This study discovered that this formulated tablet of OLE with this dose had no inverse effect on glucose metabolism related parameters and also on liver and kidney functions, but significantly improved inflammatory biomarkers which can be useful for hypertensive patients with diabetes and liver or kidney disorders.

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