Clinical Nutrition 38 (2019) 2952–2955

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Short Communication

Olive leaf tea may have hematological health benefit over green tea

F. Ferdousi [a, 1], R. Araki [b, 1], K. Hashimoto [b], H. Isoda [a, c, *]

[a] Alliance for Research on the Mediterranean and North Africa (ARENA), University of Tsukuba, Tsukuba, Japan
[b] Course of Clinical Research and Regional Innovation Donated by the Japanese Agriculture Ibaraki Public Welfare Federation, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan
[c] Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan

ARTICLE INFO

Article history:
Received 2 August 2018
Accepted 10 November 2018

Keywords:
Olive leaf tea
Olive Polyphenol
RBC
Hemoglobin
Anemia

SUMMARY

Olive leaf extracts are rich in several polyphenols having potential health benefits. We conducted the current parallel-group randomized controlled trial to compare the effects of long-term consumption of olive leaf tea (OLT) and green tea (GT) on hematological parameters in 31 female volunteers aged between 40 and 70 years of old. We found that RBC count, hemoglobin, and hematocrit were increased significantly in the OLT group than those of in the GT group at 6 and 12 weeks of intervention. Within-group comparison showed that hematocrit was significantly increased in the OLT group at 6 weeks of intervention, whereas RBC count and serum iron was significantly decreased in the GT group at 12 weeks of intervention. This is the first clinical study reporting the beneficial effects of continuous intake of OLT on hematological parameters. This observation is supported by our previous *in vitro* study reporting the differentiation-inducing effect of certain olive leaf components on human hematopoietic stem cells. However, further investigations in larger cohorts with a careful consideration of target population are required to confirm the preventive effect of OLT against anemia and other red cell disorders.

1. Introduction

Olive leaf (*Olea europaea* L. Oleaceae) contains abundant phenolic compounds, such as oleuropein (OLP), verbascoside, apigenin-7-glucoside (Api7G), luteolin-7-glucoside (Lut7G), hydroxytyrosol (HT), and tyrosol. Recent clinical and experimental evidences have suggested cardioprotective, neuroprotective, antioxidative, and hypoglycemic activities of olive leaf extract (OLE), which can be attributed to the high concentration of polyphenolic compounds in OLE [1]. Among olive polyphenols, HT and OLP have received special attention mainly for their antioxidant activities, such as scavenging oxygen and nitrogen free radicals, inhibiting LDL oxidation, and inhibiting platelet aggregation and endothelial cell activation [2]. The European Food Safety Authority has recognized protective effects of the phenolic compounds of olive on LDL oxidation [3]. Although OLP and HT may exert cytotoxic and prooxidant activity at higher doses, these, in turn, are responsible for their antiproliferative properties on several cancer cells. In addition, a recent clinical trial shows that daily supplementation of OLE rich in OLP and HT could reduce blood pressure, plasma lipids and inflammatory markers in prehypertensive male participants [4]. However, no clinical study has been performed yet to evaluate the effect of OLE on hematological parameters.

Our previous study has reported the differentiation-inducing effects of certain olive leaf phytochemicals on human hematopoietic stem cells (hHSCs) and has demonstrated their potential use in the ex vivo generation of blood cells [5]. We showed that OLP enhanced the differentiation of CD34 + cells into myelomonocytic cells and lymphocyte progenitors, whereas Api7G and Lut7G enhanced the differentiation of hHSCs towards erythroid lineages.

In order to evaluate the impact of olive leaf polyphenols on human hematological values, we conducted the current parallel-group randomized controlled trial comparing the effects of long-term consumption of olive leaf tea (OLT) and green tea (GT) on red blood cells (RBC) and hemoglobin (Hb) levels in 31 female volunteers aged between 40 and 70 years of old.

Abbreviation: OLT, olive leaf tea; GT, green tea; OLE, olive leaf extract; OLP, oleuropein; hHSCs, human hematopoietic stem cells; RBC, red blood cell; WBC, white blood cell; Plt, platelet; Hb, hemoglobin; Ht, hematocrit; SI, serum iron.

* Corresponding author. Faculty of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8572, Japan. Fax: +81 29 853 5776.
E-mail address: isoda.hiroko.ga@u.tsukuba.ac.jp (H. Isoda).
[1] These authors have contributed equally to this work.

https://doi.org/10.1016/j.clnu.2018.11.009

2. Materials and methods

2.1. Study population

The study participants were the women aged between 40 and 70 years old, who were part of our previous intervention study aiming to evaluate the effects of OLT intervention on physique, and lipid and glucose metabolism [6]. Participants were recruited through advertisements in local newspaper and in posters distributed in the university and the hospital from January 30 to April 8, 2016. The inclusion criteria of this study were: 40–70 years old female, serum LDL-cholesterol (LDL-C) level of 120–159 mg/dL, body mass index (BMI) of <30.0 kg/m^2, fasting plasma glucose level of <126 mg/dL, and HbA1c level of <6.5%. Participants were excluded from the study if they were receiving treatment for diabetes and/or other chronic diseases, and were participating in other interventional studies.

2.2. Study design

The study was a parallel-group randomized controlled trial aimed to compare the effects of OLT and GT on hematological parameters in females with borderline (120–139 mg/dL) to mildly elevated (140–159 mg/dL) serum LDL-C levels.

After baseline screening, eligible participants were allocated to OLT group and GT group using computer-generated randomization. Raw olive leaves and GT leaves (Sen-cha) were collected from Kagawa and Kyoto prefecture, respectively. Both of the tea leaves were harvested almost at the same time of the year and were primarily processed at Yamahisa Co., Ltd. (Kagawa, Japan), and Harimaen Seicha Co., Ltd. (Kyoto, Japan), respectively. Yamahisa Co., Ltd. developed a technology to get OLE having a high concentration of polyphenols in collaboration with Kagawa Prefecture Industrial Technology Center and Kagawa University Faculty of Agriculture [7]. Secondary processing of both test beverages was performed at the factory of Morita Co., Ltd. (Fukui, Japan), which obtained ISO 9001 certification. At first, 5.0 g tea leaves were extracted in 1L of boiled water. The extracts were then diluted with water at a 1:2 volume ratio. To prevent oxidation, ascorbic acid was added to the diluted solution. Finally, the solution was heat sterilized according to the food safety laws, filled in a 500 mL plastic bottle and sealed. The component composition of the test beverages is shown in Table 1.

The 12-week intervention and data collection spanned from March 26 to July 16, 2016. Each participant was instructed to drink 500 mL of test beverage twice a day, and to maintain their normal lifestyle during the intervention period. Participants were requested to record the following information daily: consumption of test beverages, stool frequency, any signs or symptoms of illnesses, use of medication, and any other complaints.

2.3. Clinical measurements

Body weight and height of the participants were measured wearing light indoor clothing without shoes. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Waist circumference was measured at the mid-point between lowest rib and iliac crest. Blood pressure was measured on the right arm using an electronic blood pressure monitor (Omron HEM-7120) with participants seated comfortably after a 5-min rest. All the data were collected at baseline, and at 6 weeks and 12 weeks of intervention.

Table 1
Nutritional composition of the test beverages in 100 g.

	Olive leaf tea	Green tea
Energy (kcal/100 g)	1	1
Total ascorbic acid (mg/100 g)	12	11
Oleuropein (mg/100 g)	42.4	n.d.
Hydroxythirosol (mg/100 g)	0.64	0.49

2.4. Blood parameters assessment

Blood samples were collected at baseline, and after 6 weeks and 12 weeks of intervention. Blood samples were taken in the morning after an overnight fast. Hematological parameters, RBC, white blood cell (WBC), platelet (Plt), Hb, hematocrit (Ht), serum iron (SI), were measured using an automated hematology system. These hematological examinations were performed at Kotobiken Medical Laboratories Inc. (Ibaraki, Japan).

2.5. Statistical analysis

The analysis was carried out on an intention-to-treat basis involving all the participants who consumed the test beverages at least once. We replaced missing data from dropouts using the baseline observation carried forward method.

Data are presented as mean ± standard deviation (SD) unless otherwise indicated. Normal distributions of the data were assessed by Shapiro–Wilk test. Log transformations were applied for the hematological parameters. Unpaired t-tests (for continuous variables) or Pearson's chi-square test (for discrete or nominal variables) were used to compare the baseline characteristics between two treatment groups. Within-group differences in the hematological parameters at different time points were compared using one-way repeated-measures ANOVA with Bonferroni's post hoc test, whereas between-group differences in the outcome parameters were assessed by unpaired t-tests. Results were considered significant at $p < 0.05$. All statistical analyses were performed using SPSS 22.0 for Windows (IBM Corp., NY, USA).

2.6. Ethical considerations

The study was approved by the University Hospital Clinical Ethics Deliberation Committee of University of Tsukuba and was conducted according to the guidelines laid down in the Declaration of Helsinki. The study was registered with trial number UMIN000020321 at University Hospital Medical Information Network-Clinical Trials Registry (http://www.umin.ac.jp/ctr) on January 11, 2016. Written informed consent was obtained from all participants before entering the study. If a participant decided to withdraw consent, the intervention was terminated.

3. Results

Figure 1 shows the flow of study participants through the recruitment and study. After baseline screening, total 32 participants were randomly allocated to OLT (n = 16) and GT (n = 16) intervention groups. Fifteen participants in each group completed the study. All analyses were conducted for 31 participants in accordance with ITT model. Overall, the study intervention was well tolerated. Total 76.7% participants reported the taste of the beverages as favorable (OLT = 66.7%, GT = 86.7%; p = 0.19). There was no significant difference in test beverage intake between the intervention groups (average intake at 12 week: OLT = 877.6 mL/day ± 258.4, GT = 982.3 mL/day ± 28.3; p = 0.16). The intervention groups did not differ significantly in age, number of postmenopausal women, weight, BMI, and blood pressure (Table 2).

There were no differences between intervention groups in any hematological parameters at the baseline and after 6- and 12-week of intervention (Supplementary table 1). However, RBC, Hb, and Ht were increased significantly in the OLT group compared with those of in the GT group at both 6 and 12 weeks from baseline (Fig. 2). SI

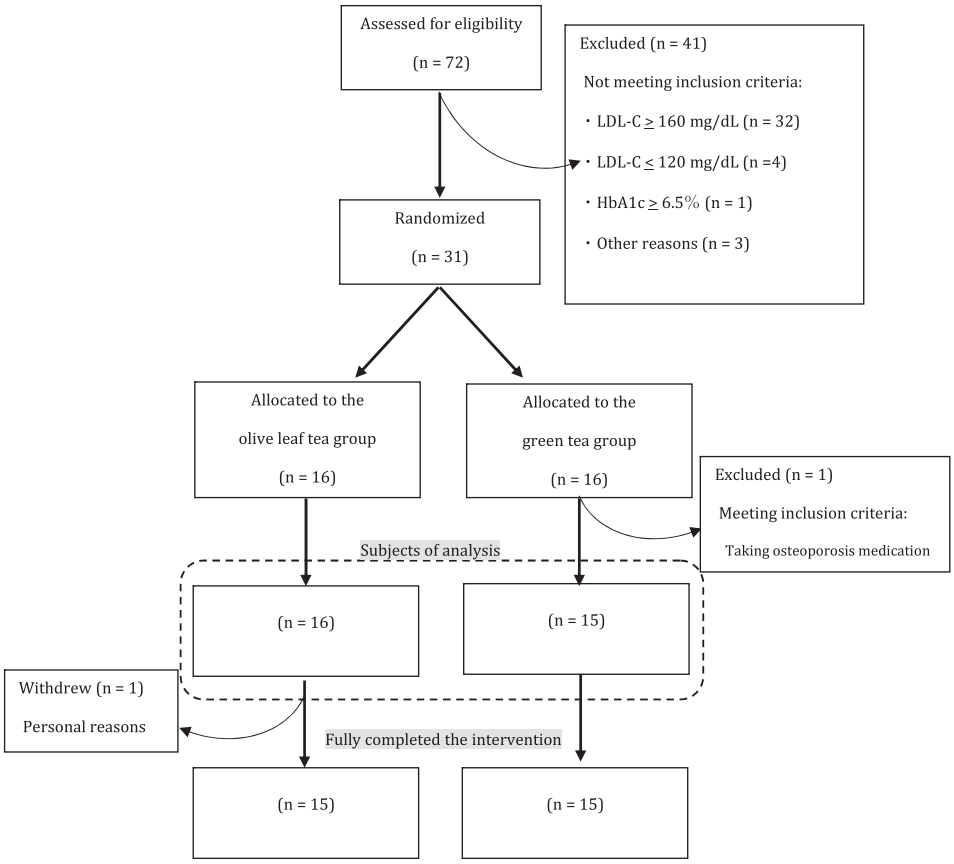


Fig. 1. Flow diagram of the study participants.

Table 2
Characteristics of the participants at baseline.

	Olive leaf tea group (n = 16)	Green tea group (n = 15)	p- value[a]
Premenopausal women, n (%)	5 (31.3)	6 (40.0)	0.59[b]
Age (years)	54.9 ± 7.7	54.9 ± 7.9	0.98
Body weight (kg)	52.2 ± 7.5	54.1 ± 6.3	0.45
BMI (kg/m^2)	20.9 ± 3.1	21.9 ± 2.7	0.38
Waist circumference (cm)	78.1 ± 9.1	80.6 ± 6.1	0.37
Systolic Blood Pressure (mmHg)	115.9 ± 19.4	127.7 ± 20.8	0.11
Diastolic Blood Pressure (mmHg)	78.4 ± 11.3	82.6 ± 12.7	0.33

Values are mean ± SD.
[a]p-values are assessed between the olive leaf tea group and the green tea group by unpaired t-test.
[b] p-values are assessed between the olive leaf tea group and the green tea group by Pearson's chi-square test.

was decreased in both intervention groups, however, the decrease rate was higher in GT group than in OLT group ($p = 0.059$).

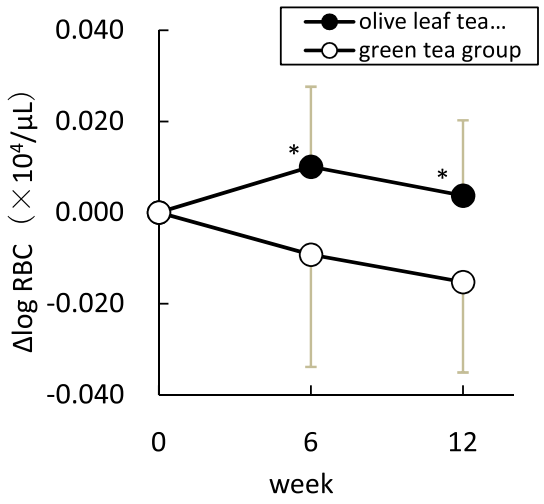
With-in group comparison by repeated measure one-way ANOVA showed that Ht was significantly increased in the OLT group after 6 weeks of intervention ($p < 0.01$). On the other hand, RBC count was significantly decreased at 12 weeks from baseline ($p < 0.05$) in the GT group. SI was also significantly decreased at both 6 weeks ($p < 0.05$) and 12 weeks ($p < 0.01$) of intervention in the GT group.

4. Discussion

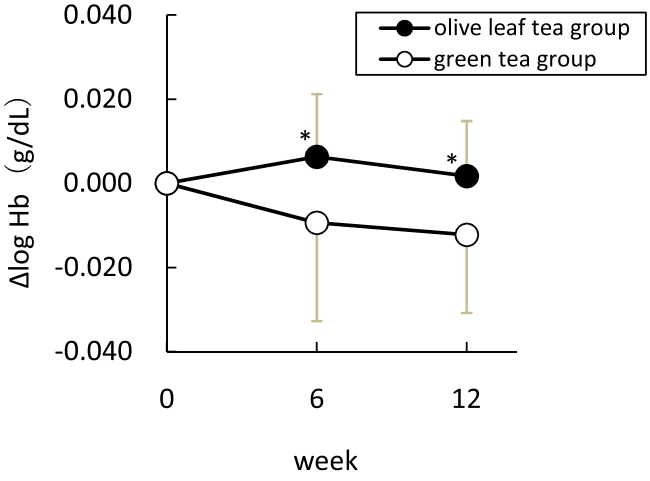
The present study indicated that long-term intake of OLT may have favorable effects on human hematological parameters. We found that levels RBC count, Hb, and Ht were significantly increased in OLT group compared with those of in GT group. We reported in previous *in vitro* study that certain olive leaf components, namely Api7G and Lut7G induces hHSCs differentiation towards erythroid lineage, thus, have potential of ex vivo generation of blood cells [5].

We found that SI was significantly decreased in the GT group after 6 and 12 weeks of intervention. Previous study reported that certain phenolic components of GT interfere with the nonheme-iron absorption in young females [8]. Unlike OLT, GT contains tannins and caffeine that were reported to inhibit iron absorption [9]. Therefore, we can assume that OLT is less likely to cause anemia. In our study, no participant was anemic at the baseline. And, there was no significant difference in any hematological index between pre- and post-menopause participants at baseline and after 6 and 12 weeks of OLT intervention. Thus, although our present results suggest potential hematological health benefit of OLE, we could not confirm its preventive effect against anemia.

Anemia is the most common blood disorder, affecting about one third of the global population [10]. Certain groups of individuals are at a higher risk for anemia, including women at puberty and child bearing age, during pregnancy and after delivery, children, and the elderly where blood loss or Iron-deficiency from poor nutrition are most common causes of anemia. In recent years, there has been



A: Effect of olive leaf tea on RBC count



B: Effect of olive leaf tea on hemoglobin

Fig. 2. A and B: Effect of olive leaf tea on RBC count and hemoglobin (*p < 0.05, unpaired t-test).

growing interest in the use of nutraceuticals in the form of complementary and alternative medicines derived from naturally occurring resources. OLT may be a promising candidate for a natural approach to prevent and cure anemia and other red cell disorders. However, further investigations in larger cohorts with a careful consideration of target population are warranted to confirm the preventive effect of OLT against anemia.

Conflicts of interest

RA, KH, and HI received research funds of joint research from Yamahisa Co., Ltd.

Funding sources

"Subsidies for strengthening functional food industry" by Kagawa Prefecture (2015).

CRediT authorship contribution statement

F. Ferdousi: Formal analysis, Validation, Visualization, Writing - original draft. **R. Araki:** Conceptualization, Funding acquisition, Investigation, Data curation, Formal analysis, Visualization, Writing - original draft. **K. Hashimoto:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing - review & editing. **H. Isoda:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing - review & editing.

Acknowledgements

This work was supported by Kagawa Prefectural grant program for "Subsidies for strengthening functional food industry" in 2015, the fund for joint research with Yamahisa Co., Ltd., and the Center of Innovation Program from Japan Science and Technology Agency (JST), Japan. We thankfully acknowledge Keiko Fujie, Yoshio Nakata, and Hiroaki Suzuki for the performance of the studies, and Katsutaro Uematsu, and Koichi Matsui for test beverage preparation, and Hiroyuki Shibasaki for component analysis of test beverage.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2018.11.009.

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