Nor. Afr. J. Food Nutr. Res. 2024; 8 (17): 86 – 98

https://doi.org/10.51745/najfnr.8.17.86-98

https://www.najfnr.com

ORIGINAL ARTICLE

Functional and Novel Foods
Human and Clinical Nutrition

Randomized, placebo-controlled pilot study investigating the effects of *Laurus nobilis* tea on lipid profiles and oxidative stress biomarkers in healthy North African volunteers

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ABSTRACT

Background. *Laurus-nobilis* (Ln) is an evergreen tree from the Lauraceae family, widely utilized for its culinary and medicinal purposes. **Aims.** This study aims to investigate the effects of Ln-tea (Lnt) consumption on lipid profiles and oxidant/antioxidant stress biomarkers in healthy volunteers. **Methods.** A randomized, double-blind, placebo-controlled trial (PACTR202205671550114) was conducted involving healthy volunteers. Participants (n=62) were randomly assigned to either the experimental-group (EG, n=31), receiving Lnt, or the control-group (CG, n=31), receiving a placebo tea. The regimen was administered once daily for ten consecutive days. Blood samples were collected from each participant on two occasions: one day before the study (Day1) and one day after its completion (Day11). These samples underwent analysis for lipid data, including cholesterol, triglycerides, high- and low- density-lipoprotein-cholesterol (HDL-C, LDL-C, respectively), and low-density-lipoprotein-receptor (LDL-R). Additionally, oxidant/antioxidant stress biomarkers, such as superoxide-dismutase, uric-acid, and carbonylated-proteins, were assessed. **Results.** Data from seven participants (one from the EG, and six from the CG) were excluded from the final statistical analysis, resulting in 55 volunteers completing the study (30 in the EG, 25 in the CG). The two groups exhibited comparable demographic and clinical characteristics. In the EG, LDL-C decreased by 0.42 while HDL-C and LDL-R increased by 0.18 mmol/L and 189.45 pg/mL, respectively, compared to Day1. On Day11, the EG displayed lower LDL-C value and higher values of HDL-C and LDL-R compared to the CG. Significant interactive effects of the groups (2) vs. days (2) were observed for LDL-C, HDL-C, and LDL-R. No significant changes in oxidant/antioxidant stress biomarkers were noted between Day1 and Day11 in both groups. However, the EG showed higher levels of superoxide-dismutase compared to the CG on Day 11. Significant interactive effects of the groups (2) vs. days (2) were noted for superoxide-dismutase and carbonylated-proteins. **Conclusions.** Lnt infusion showed potential in modulating LDL-R activity levels, accompanied by elevated antioxidant activity.
Keywords: Laurus, oxidative stress, plant extracts, therapeutic use, tea.

ARTICLE INFORMATION

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Received: December 03, 2023
Revised: March 09, 2024
Accepted: March 13, 2024
Published: April 04, 2024

Article edited by:
Pr. Meghit Boumediene Khaled

Article reviewed by:
Pr. Mustapha Diaf
Dr. Hocine Remini

Cite this article as: Chbili, C., Mrad, S., Graiet, H., Selmi, M., Maatoug, J., Maoua, M., Abdellaoui, L., Mrizek, N., Nouira, M., Ben Fredj, M., & Ben Saad, H. (2024). Randomized, placebo-controlled pilot study investigating the effects of Laurus nobilis tea on lipid profiles and oxidative stress biomarkers in healthy North African volunteers. *The North African Journal of Food and Nutrition Research, 8* (17): 86 – 98. https://doi.org/10.51745/najfnr.8.17.86-98

1 Introduction

Laurus nobilis (Ln), an evergreen tree belonging to the Lauraceae family, holds a notable position in both culinary and medicinal domains [1]. The origins of this plant are believed to rooted in the Mediterranean region and Asia [1]. In North Africa, particularly in Tunisia, Ln is referred to as "*Rand*", and it thrives abundantly along riversides, mountains, and wet cliffs [2]. The prevalence of "*Rand*" tree is particularly notable in humid and sub-humid bioclimatic

zones, with a significant presence in northwestern areas such as Ain Draham, Tabarka, Kef, and Cap-Bon [2]. Ln holds significance as a species and an aromatic substance of industrial relevance, finding applications in the food, pharmaceuticals, and cosmetics sectors [3,4]. Both the fresh plant and its dried leaves are extensively utilized in culinary practices and food preservation [3,4]. In the realm of herbal medicine and *in vitro* research, Ln has gained recognition for its antibacterial, antifungal, anti-diabetic, anti-hyperlipidemic, and anti-inflammatory properties [5,6]. Consequently, it has been harnessed in the treatment of various conditions, including rheumatic diseases, skin rashes, and gastrointestinal issues [4]. The leaves of Ln have been traditionally utilized for managing neurological disorders such as epilepsy, neuralgia, and parkinsonism [3, 4]. Furthermore, Ln has served various medicinal purposes, acting as a carminative, astringent, diaphoretic, emetic, diuretic, emmenagogue agent, anti-stress and anxiolytic effects and in the prevention of migraines [6, 7].

A preceding investigation highlighted the abundant presence of chemical compounds in Ln with notable antioxidant activities, including scavenging activity [8]. Ku et al. [9]. have reported that flavonoid compound as a candidate therapeutic agent for the treatment of vascular inflammatory diseases. In this case, it includes compounds such as sesquiterpene, flavonoids composition of the methanolic extract Ln are the main responsible of such kind of activity including inhibitory effects on nitric oxide production [10-12]. Spirafolide and costunolide, which were found in Ln [12, 13], can be used in the treatment and prevention of various diseases. Building on this, the hypothesis was posited that the intake of Ln tea (Lnt) infusion could yield significant benefits, manifesting as alterations in peripheral biomarkers [14]. Furthermore, the consumption of dried aqueous extracts of Ln was identified to enhance glucose and insulin metabolism, as well as modulate circulating blood lipids, particularly in individuals with type 2 diabetes mellitus [14]. Traditional medicine recognizes Ln as a medicinal plant widely used in treating various conditions, such as respiratory, diabetic, and digestive disorders [15]. In line with Tunisian tradition, Ln is commonly administered in the form of an infusion derived from the fresh or dried plant [2]. Notably, no scientific data, to the authors' best knowledge, has explored the therapeutic effects of Lnt infusion on healthy individuals, particularly in terms of its influence on blood lipid data and oxidant/antioxidant stress biomarkers—a domain of interest in cardiovascular disease prevention [11, 12]. Numerous randomized clinical trials (RCT) have underscored the role of low-density lipoprotein cholesterol (LDL-C) levels in reducing the risk of cardiovascular events through the upregulation of the low-density lipoprotein receptor (LDL-R) [16, 17]. The LDL-R serves as the primary pathway for cholesterol removal from circulation, with its activity intricately regulated by intracellular cholesterol levels [18]. Studies have indicated that LDL-R internalizes LDL-C from plasma, releasing cholesterol into the cell, and appears pivotal in the feedback inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase [19]. Reduced LDL-R activity in the general population often contributes to hypercholesterolemia, fostering the development of atherosclerosis over time [20]. In the realm of biology, oxidative reactions can inflict damage on various cellular components, a phenomenon associated with aging and specific diseases, including cardiovascular diseases [11, 12].

The primary objective of this double-blind RCT, encompassing two groups [experimental (EG) and control (CG) groups], was to scrutinize the impacts of Lnt consumption on lipid data and oxidant/antioxidant stress biomarkers in healthy individuals. The null hypothesis posited that both groups would exhibit comparable LDL-R values ten days post-intervention.

2 Population and Methods

The present study consisted of two distinct components. The primary objective, or the aim of this investigation, was to assess the impact of Lnt consumption on lipid parameters and oxidant/antioxidant stress biomarkers in healthy individuals. The secondary aspect aimed to evaluate the influence of Lnt consumption on the Neutrophils/Lymphocytes ratio and various elements of complete blood count.

2.1 Study design

Our double-blind RCT spanned seven months from August 2019 to February 2020. The study took place at the Department of Occupational Medicine, Farhat Hached Hospital, Sousse, Tunisia. Approval was obtained from the Medical Research and Ethical Committee at the Faculty of Medicine of Sousse (Approval N° CEFMS 29/2019), and written consent was obtained from all participating volunteers. All medical examinations were provided to the volunteers free of charge, and they received reports detailing the findings of their explorations. Additionally, the trial was registered in the Pan African Clinical Trial Registry database (www.pactr.org; PACTR202205671550114).

2.2 Study population

Volunteers were recruited through three distinct avenues: Firstly, 60 interns/residents and medical staff members of the Occupational Medicine Department were invited by a senior author (MM as listed among the authors); secondly, 50 interns/residents of the Laboratory of Biochemistry (Farhat Hached Hospital, Sousse, Tunisia) were invited by a senior

author (SM as listed among the authors); and thirdly, an announcement was disseminated within the Occupational Medicine department inviting accompanying persons to participate.

The inclusion criteria comprised individuals who were non-smokers, adhered to a non-vegetarian diet, were not pregnant, did not take any medication or nutritional supplements, did not have chronic pathologies such as cardiovascular diseases, diabetes-mellitus, arterial hypertension, asthma, psychiatric disorders, and gastrointestinal issues), and no food allergy. Exclusion criteria involved individuals who were absent during the second visit (Day11) or did not consume Lnt or placebo tea at least once during the 10 – day study period.

2.3 Sample size

The null hypothesis [21] was H0: m1 = m2 and the alternative hypothesis was Ha: m1 = m2 + d, where "d" is the difference between two means, and n1 and n2 are the sample sizes for the EG and the CG, such the total sample (N) = n1 + n2. The sample size was estimated using the following formula [21]:

$$N = (r + 1)(Z\alpha/2 + Z1 - \beta)2\delta2]/r\,d2$$

Where:

- "Zα/2" is the normal deviate at a level of significance = 2.58 (1% level of significance);
- "Z1-β" is the normal deviate at 1-β% power with β% of type II error (1.64 at 95% statistical power);
- "r" (=n1/n2) is the ratio of sample size required for the two groups (r = 1 gives the sample size distribution as 1:1 for the two groups);
- "s" and "d" are the pooled standard deviation and difference of LDL-R means of the two groups. Given the pioneer character of the present study, the latter two values were arbitrarily fixed by the authors at 600 and 400 pg/mL for the EG and the CG, respectively, with a common standard deviation equal to 250 pg/mL.

The sample size for the study consisted of 56 volunteers with 28 individuals allocated to each group. Anticipating a 10% attrition rate, accounting for potential absences during the second visit and non-consumption of either Lnt- or placebo-tea. The corrected total sample size was adjusted to 62 volunteers, calculated as 62 = 56 / (1 - 0.10), resulting in 31 participants in each group.

2.4 Random allocation and blinding

Volunteers were randomly allocated to either the EG receiving Lnt once a day for 10 consecutive days, or the CG receiving placebo tea once a day for 10 consecutive days. Randomization was conducted using a pre-defined sequence generated by Random Allocation software, version 1.0.0 (M. Saghaei, Isfahan University of Medical Sciences, Isfahan, Iran). Throughout the study, blinding was maintained, with both volunteers and investigators kept unaware of group assignments. A non-study physician concealed the allocation sequence, and group assignments were revealed to investigators only after blood results were obtained.

2.5 Applied protocol and examinations performed

Figure 1 depicts the study protocol, involving two visits with a 10 – day interval. Volunteers were instructed to adhere to their regular dietary habits and physical activity levels throughout the study period. They were instructed to refrain from consuming any herbal recipes or nutritional supplements that could potentially affect metabolism during the study. The first visit (Day1) involved completing a standard medical questionnaire, measuring body weight, conducting a physical examination, collecting blood sample, and dispensing ~~of~~ tea (Lnt or placebo). The second visit (Day11), conducted 10 days after tea consumption, involved an assessment of tolerability to Lnt and another blood sample collection.

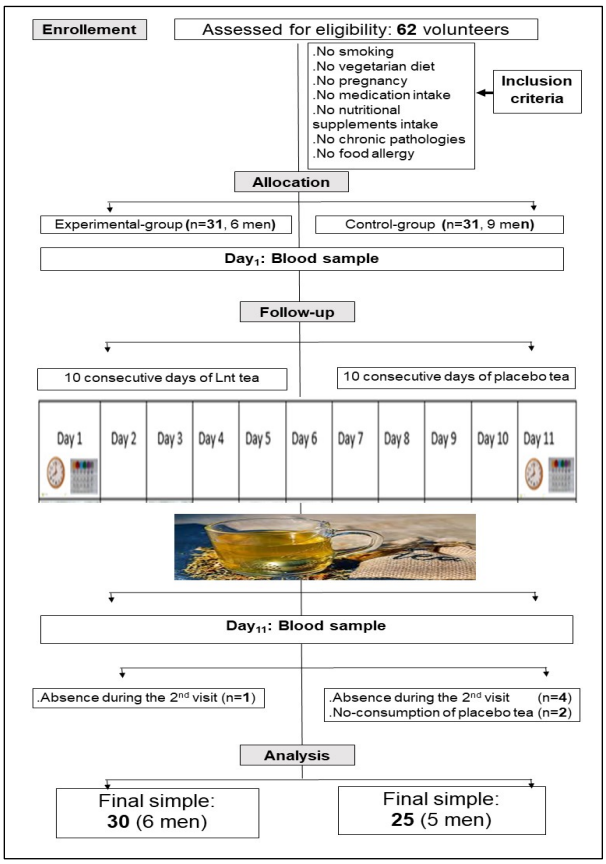

Figure 1. Study flowchart

Blood sample: Oxidant/antioxidant stress biomarkers (superoxide-dismutase, uric-acid, carbonylated-proteins and lipid data (cholesterol, triglycerides, low- and high- density lipoprotein cholesterol, low-density lipoprotein-receptor)

Blood samples, collected from a peripheral arm vein after an overnight fast, were promptly centrifuged (10 min, 3000 rpm, 4 °C) for subsequent analysis. Assessment of antioxidant stress status involved the determination of superoxide-dismutase levels [22] as well as uric-acid concentrations [23, 24]. Superoxide-dismutase levels were quantified using enzyme-linked immunosorbent assay (ELISA) kits obtained from Abcam systems, employing a quantitative sandwich enzyme immunoassay technique (with a coefficient variation (CV) of 5.1%). Protein oxidation levels, expressed as nmol of carbonyl/mg of protein, were determined in plasma samples employing the carbonylation-protein assay method [25]. LDL-R was determined using ELISA kits from R&D systems (with a CV of 2%) to further investigate the plant's effect on lipid parameters. Plasma concentrations of total cholesterol (with a CV of 3%), triglycerides (CV < 5%), high-density lipoprotein cholesterol (HDL-C) (CV = 5%), and uric-acid (CV < 2%) were measured using enzymatic assays on an automated system (DX600, Beckman Coulter). LDL-C levels were calculated using the Friedewald formula when triglyceride values were less than 3.75 mmol/L [LDL-C = total cholesterol – (HDL-C + 0.45 triglycerides)] [26].

2.6 Phytochemical composition of Ln

The phytochemical composition of Ln encompasses essential oils abundant in compounds such as eucalyptol (1,8-cineole), α-pinene, β-pinene, and sabinene [27]. Moreover, it harbors sesquiterpenes such as humulene and caryophyllene, as well as tannins, flavonoids, and other phenolic compounds [27]. Additionally, Ln contains terpenoid lactones such as lauroside B and lauroside C [27]. These phytochemicals contribute to the plant's aroma, flavor, and potential health benefits [27].

2.7 Herbal material

Volunteers were instructed to adhere to a regimen of either Lnt, for the EG or placebo tea, for the CG, to be consumed daily at 7 pm for a duration of 10 consecutive days. They were strongly encouraged to maintain a consistent daily routine, encompassing both food intake and exercise activities.

For the EG, volunteers were provided detailed instructions on the preparation of Lnt. This involved infusing five grams of dried Lnt leaves in 100 mL of boiling water for 15 minutes, followed by filtering the mixture through a strainer. The prescribed daily dosage of Lnt was set at five grams, corresponding to the weight of two spoons, in accordance with traditional usage [5]. Consistent with the methodology outlined by Khan et al. [14], a 10-day study period was considered methodologically appropriate. The *Laurus nobilis* leaves utilized in the study were sourced from Hammam Jedidi-Mount, Zaghouen (a sub-humid area) between March and May 2019. Authentication of the plant material was performed by an herbarium doctor at the Higher School of Agriculture of Mograne, Zaghouan, Carthage University, adhering to the flora of Tunisia [2]. Fresh leaves were stored at room temperature, shielded from sunlight, in a well-ventilated room until they were thoroughly dried. Subsequently, five grams of dried Ln leaves were employed for the infusion in 100 mL of boiling water. Following a steeping period of 15 minutes, the infusion underwent filtration and was stored at 20°C for a month until subjected to chromatographic analysis. The Lnt preparation was utilized for preliminary high-performance liquid chromatography and mass spectrometry experiments.

The placebo material utilized in the study was obtained from the robust stems of *Origanum syriacum* L. [28]. These stems underwent a meticulous preparation process, involving thorough cleaning followed by boiling for a duration of five hours. During this process, the water was changed on an hourly basis to minimize the content of water-soluble component [28].

2.8 Statistical analysis

Quantitative data, confirmed to exhibit normal distribution via the Kolmogorov-Smirnov test, were presented as mean ± standard deviation (95% confidence interval), while the distribution of sex was expressed as a relative number (%). Regarding biological data, ΔData denoted the disparity between values observed on Day11 and Day1 (ΔData = Day11 minus Day1).

Various comparisons were conducted: a two-sided chi-2 test was employed to compare the percentage of women between the two groups, a Student's t-test was utilized to compare biological data of i) Day1 and Day11 within each group, ii) The EG and the CG for the same day, and iii) ΔData of the EG and the CG. Furthermore, factorial analysis of variance was employed to analyze the highest order interactive effects of multiple categorical independent factors (groups 2 vs. days 2). Hedge's LDL-R value served as the effect size measurement [29], with an effect size of ≤ 0.2 denoting a small effect, approximately 0.5 a medium effect, roughly 0.8 a large effect, and exceeding 1.30 a very large effect. The significance level for all tests was set at $p < 0.05$.

3 Results

3.1 Characteristics of healthy volunteers

Sixty-two healthy volunteers were initially recruited for the study, with 31 assigned to each group. However, data from seven volunteers (one from the EG and six from the CG) were deemed ineligible for inclusion in the final statistical analysis. Consequently, 55 volunteers successfully completed the study with 30 in the EG, and 25 in the CG. The two groups

demonstrated identical distributions concerning sex, age, and body weight, and no significant deviations from the study protocol were recorded during the 10 – day tea infusion period (Table 1). Additionally, comparable baseline values for lipid data and oxidant/antioxidant stress biomarkers were observed in both groups on Day1.

Table 1. Characteristics and initial biological data of the healthy volunteers: Experimental-group (EG, n=30) and control group (CG, n=25)

Data	Category/unit	EG	CG	p-value
Sex and anthropometric data				
Sex	Women	26 (86.66)	20 (80.00)	0.5062
Age	year	37 ± 9 (34 to 41)	36 ± 9 (33 to 40)	0.6429
Weight	kg	68 ± 9 (65 to 75)	70 ± 12 (65 to 72)	0.5731
Initial lipid data				
Triglycerides	mmol/L	1.15 ± 0.54 (0.79 to 1.27)	1.03 ± 0.63 (0.93 to 1.37)	0.4470
Cholesterol	mmol/L	4.21 ± 1.08 (4.30 to 4.87)	4.58 ± 0.77 (3.76 to 4.66)	0.1431
LDL-C	mmol/L	2.79 ± 0.72 (2.50 to 3.09)	2.79 ± 0.79 (2.49 to 3.09)	0.9972
HDL-C	mmol/L	1.27 ± 0.29(1.16 to 1.38)	1.31 ± 0.29 (1.19 to 1.43)	0.6887
LDLR	pg/mL	413.89 ± 200.97 (354.54 to 534.55)	444.55 ± 241.03 (330.94 to 496.85)	0.6150
Initial oxidant/antioxidant data				
Superoxide-dismutase	U/mg	72.33 ± 26.40 (62.47 to 82.19)	73.85 ± 28.01 (62.28 to 85.41)	0.8373
Uric-acid	µmol/L	261.38 ± 90.50 (227.59 to 295.17)	237.76 ± 74.88 (206.85 to 268.67)	0.3026
Carbonylated-proteins	nmol/mg protein	0.86 ± 0.15 (0.84 to 0.97)	0.91 ± 0.17 (0.80 to 0.93)	0.3254

HDL-C: High-density lipoprotein cholesterol. LDL-C: Low-density lipoprotein cholesterol. LDLR: Low-density lipoprotein-receptor.
Quantitative data were mean ± standard deviation (95% confidence interval), and sex was number (%). P-value: Student t test or 2-sided chi-2 test between the 2 groups.

Table 2. Lipid data of the healthy volunteers: Experimental-group (EG, n=30) and control-group (CG, n=25)

Data	Unit	Groups	Day_1	Day_{11}	Δ	p-value: Day_1 vs. Day_{11}	Factorial ANOVA
Triglycerides	(mmol/L)	**EG**	1.03 ± 0.63	0.94 ± 0.54	-0.09 ± 0.26	0.0591	F (1.106) =0.1284
		CG	1.15 ± 0.54	1.14 ± 0.66	-0.01 ± 0.72	0.9318	p = 0.7207
		p-value: EG vs. CG	0.4470	0.2125	0.5648	-	-
Cholesterol	(mmol/L)	**EG**	4.58 ± 0.77	4.49 ± 0.80	-0.09 ± 0.30	0.1121	F (1.106) =0.2725
		CG	4.21 ± 1.08	4.31 ± 1,14	0.100 ± 1.40	0.7249	p = 0.6026
		p-value: EG vs. CG	0.1431	0.4872	0.4737	-	-
LDL-C	(mmol/L)	**EG**	2.79 ± 0.79	2.37 ± 0.69	-0.42 ± 0.48	0.0001	F (1.106) =4.7653
		CG	2.79 ± 0.72	2.98 ± 0,71	0.19 ± 0.9967	0.3540	p = 0.0312¥
		p-value: EG vs. CG	0.9972	0.0022*	0.0043*	-	-
HDL-C	(mmol/L)	**EG**	1.27 ± 0.29	1.45 ± 0.32	0.18 ± 0.32	0.0265	F (1, 106) =5.9914
		CG	1.31 ± 0.29	1.19 ± 0.38	-0.12 ± 0.30	0.0529	p = 0.0160¥
		p-value: EG vs. CG	0.5823	0.0085*	0.0008*	-	-
LDLR	(pg/mL)	**EG**	444.55±241.04	634.01±290.20	189.45±238.66	0.0001	F (1.106) =4.4305
		CG	413.90±200.97	410.80±199.15	-3.09±7.68	0.0551	p=0.0377¥
		p-value: EG vs. CG	0.6150	0.0020*	0.0002*	-	-

ANOVA: Analysis of variance. **HDL-C**: High-density lipoprotein cholesterol. **LDL-C**: Low-density lipoprotein cholesterol. **LDLR**: low-density lipoprotein-receptor. **Δ**: Day_{11} value minus Day_1 value. Data were mean ± standard deviation. *P-value (Student test between the 2 groups for the same day of for Δ) < 0.05. ¥P-value (Factorial ANOVA between the 2 days for the 2 groups) <0.05.

3.2 Impact of Lnt consumption on blood lipid data

Table 2 presents the lipid data for both groups, revealing the following key findings:

- In the EG, LDL-C exhibited a significant decrease of 0.42 mmol/L at Day11 compared to Day1, while HDL-C and LDL-R values showed significant increases of 0.18 mmol/L and 189.45 pg/mL, respectively. No statistically significant changes were observed in the CG;
- On Day11, the EG exhibited a lower LDL-C value and higher values of HDL-C and LDL-R compared to the CG. The effect size for LDL-R was large (Hedges' unbiased d = + 0.869);
- Significant interactive effects of the groups (2) vs. days (2) were observed for LDL-C, HDL-C, and LDL-R.

3.3 Impact of Lnt consumption on oxidant / antioxidant stress biomarkers

Table 3 outlines the oxidant/antioxidant stress biomarkers for both groups, yielding the following primary conclusions:

- No statistically significant changes were observed between Day1 and Day11 in either group.
- On Day11, the EG displayed a higher value of superoxide-dismutase compared to the CG.
- Significant interactive effects of the groups (2) vs. days (2) were noted for superoxide-dismutase and carbonylated-proteins.

4 Discussion

The present investigation elucidates significant effects of Lnt consumption on lipid parameters, including LDL-C, HDL-C, and LDL-R, as well as on oxidant/antioxidant stress biomarkers, such as superoxide-dismutase and carbonylated-proteins. The rejection of our null hypothesis, which postulated comparable LDL-R values between the two groups following 10 days of intervention, underscores the potential of Lnt infusion to positively influence LDL-R activity, alongside notable antioxidant effects. This study represents the inaugural double-blind, placebo-controlled RCT exploring the impact of Lnt infusion on lipid profiles and oxidant/antioxidant stress biomarkers.

4.1 Impact of Lnt consumption on blood lipid data

Lnt consumption exerted a beneficial impact on HDL-C, LDL-C, and LDL-R (Table 2). Following a 10 – day regimen of Lnt intake, there was a notable increase in HDL-C level by 0.18 mmol/L and a corresponding decrease in LDL-C level by 0.42

Table 3. Oxidant/antioxidant biomarkers of the healthy volunteers: Experimental-group (EG, n=30) and control-group (CG, n=25)

Data	Unit	Groups	Day_1	Day_{11}	Δ	p-value: Day_1 vs. Day_{11}	Factorial ANOVA
Superoxide dismutase	(U/mg)	**EG**	72.33±26.40	87.24±37.89	14.91±29.69	0.0822	F (1. 106) = 5.1837, p=0.0248¥
		CG	73.85±28.01	63.47±18.83	-10.38±28.01	0.1307	
		p-value: EG vs. CG	0.8373	0.0061*	0.0021*	-	-
Uric-acid	(µmol/L)	**EG**	261.38±90.50	224.98±68.02	-36.40±91.96	0.0835	F (1. 106) = 1.3810 p=0.2425
		CG	237.76±74.88	236.72±78.55	-1.036±70.13	0.9417	
		p-value: EG vs. CG	0.3026	0.5550	0.1207	-	-
Carbonylated proteins	(nmol/mg protein)	**EG**	0.91±0.17	0.82±0.19	-0.09±0.26	0.0672	F (1.106) = 3.9408, p=0.0497¥
		CG	0.86±0.15	0.90±0.17	0.04±0.22	0.3808	
		p-value: EG vs. CG	0.3254	0.0809	0.0543	-	-

ANOVA: analysis of variance. **Δ**: Day_{11} value minus Day_1 value. Data were mean ± SD. *p-value (Student test between the 2 groups for the same day of for Δ) < 0.05. ¥p-value (Factorial ANOVA between the 2 days for the 2 groups) < 0.05.

mmol/L were observed. The elevation in HDL-C levels assumes significance owing to its recognized cardioprotective attributes [5,30]. Additionally, HDL-C may play a protective role against atherogenic diseases by inhibiting the formation of oxidatively modified LDL-C [31]. Moreover, HDL-C exhibits a protective effect against atherosclerosis by impeding the accumulation of lipid peroxides on LDL-C [32]. These findings align with a previous clinical trial that reported a significant increase in HDL-C levels in diabetic patients consuming capsules containing powdered Lnt [30]. The reduction in LDL-C levels subsequent to Lnt consumption aligns with the observations of Khan et al. [30], who documented a significant decrease in LDL-C levels in diabetic patients following Lnt intake.

To further elucidate the mechanism underlying the decrease in LDL-C levels subsequent to Lnt consumption, our study investigated the effect of this plant on LDL-R levels. The results suggested that Lnt infusion might enhance LDL-R levels (refer to Table 2). Based on these findings, it is plausible to hypothesize that Lnt reduces LDL-C concentrations by increasing LDL-R levels. In existing literature, it is well-established that LDL-R facilitates the clearance of LDL-C from the bloodstream [33]. Therefore, enhancing LDL-R levels represents a promising therapeutic avenue for managing hypercholesterolemia [34]. Kinetic studies have demonstrated that the induction of LDL-R activity is a major mechanism through which statin treatment reduces circulating atherogenic LDL-C levels in humans [35].

Numerous RCTs consistently highlight pivotal role of reduced LDL-C levels in mitigating cardiovascular risk through the upregulation of LDL-R [16, 17]. This observed increase in LDL-R expression is frequently associated with the inhibition of cholesterol synthesis, accomplished by the suppression of HMG-CoA reductase. Consequently, this cascade stimulates the LDL-R pathway, augmenting the binding capacity of LDL-C to receptors on hepatocyte cell surfaces and ultimately lowering circulating LDL-C levels in the bloodstream [20], In this context, it is proposed that Lnt may modulate HMG-CoA reductase by inducing LDL-R expression, thus constituting a significant mechanism in the reduction of LDL-C levels. Additionally, previous studies have reported the potent cholesterol-lowering activity of an ethanolic extract of Lnt leaves attributed to its inhibitory effect on HMG-CoA reductase and its favorable impact on lipid profile [36].

In the current study, following Lnt consumption, we noted a non-significant reduction of 0.09 mmol/L in triglyceride levels within the EG (Table 2). This reduction was found to be correlated with a higher LDL-R plasma level, aligning with results reported in a previous study [16]. The transportation of triglycerides from the liver to peripheral tissues primarily occurs through very low-density lipoproteins containing apolipoprotein B100 [16]. Building on these observations, it is postulated that the ingestion of Lnt infusion increases LDL-R activity, thereby facilitating the clearance of triglycerides due to their affinity for apolipoprotein B100, an inherent component of triglyceride transporter molecules presents in very low-density lipoproteins [16]. It is essential to underscore that the primary aim of our study was to ascertain the effects of Lnt on LDL-R levels, with the investigation refraining from delving into the mechanistic underpinnings of these effects.

4.2 Impact of Lnt consumption on oxidant/antioxidant stress biomarkers

The ingestion of Lnt demonstrated a beneficial influence on key oxidant/antioxidant stress biomarkers, specifically superoxide-dismutase and carbonylated-proteins, as outlined in Table 3. Lnt consumption increased superoxide-dismutase activity, thereby contributing to a reduction in reactive oxygen species levels. Superoxide-dismutase enzymes play a crucial role in catalyzing the dismutation of superoxide radicals into hydrogen peroxide and molecular oxygen, serving as a vital defense mechanism against the toxicity of superoxide radicals [22]. This finding aligns with previous studies highlighting the antioxidant effects of Lnt extract [37]. For instance, Gazoui et al. [38] demonstrated the promotive effect of Lnt superoxide-dismutase activity against oxidative damage induced by lead in rat brains, while Casamassima et al. [39] reported improved superoxide-dismutase activity in rabbits administered Lnt. Moreover, the ingestion of Lnt resulted in a reduction in oxidative damage, manifested a decline in the levels of carbonylated-proteins (Table 3). The latter serve as indicators of protein oxidative damage and are implicated in the generation of reactive oxygen species [40], with their accumulation associated with various diseases, including atherosclerosis [41]. Previous studies have reported a decrease in carbonylated-proteins with Lnt consumption, implying its protective role against oxidative damage [37, 38].

While the consumption of Lnt demonstrated a trend towards reducing uric-acid levels by 36 µmol/L (p=0.0835) (Table 3), statistical significance was not attained. This lack of significance may be attributed to the normal uric-acid levels observed in the study participants (< 530 µmol/L in women and < 7.0 µmol/L in men) [42]. Consequently, the decrease in uric acid levels may not be deemed clinically significant compared to that observed in the general population [24]. Uric-acid, an end product of purine metabolism, can act as a pro-oxidant, especially at elevated concentrations, thereby serving as a potential biomarker of oxidative stress [23]. However, at physiological levels, uric-acid may exert therapeutic effects as an antioxidant [43] by scavenging free radicals and stabilizing

ascorbate in biological fluids [44]. Notably, uric-acid prevents the inactivation of superoxide-dismutase during co-incubation with hydrogen peroxide at physiological levels [43]. Additionally, in mice models, superoxide-dismutase activity can be restored by elevating the uric-acid levels within the activated superoxide-dismutase due to apolipoprotein E-deficiency [43]. Yasser et al. [45] reported that Lnt consumption reduces xanthine-oxidase activity. Indeed, xanthine-oxidase catalyzes the metabolism of hypoxanthine to xanthine, and subsequently xanthine to uric-acid [12]. This metabolic process yields superoxide anion and hydrogen peroxide, leading to oxidative damage of living tissues [12]. Therefore, the inhibition of xanthine oxidase by Lnt may hold therapeutic potential for various pathological conditions, such as gout and post-ischemic tissue injury which are attributed to the generation of uric-acid and superoxide anion radicals [46, 47]. Our results are consistent with the aforementioned findings [46,47]. The observed decrease in uric-acid could be attributed to these mechanisms associated with Lnt consumption. Several studies have highlighted the association between elevated uric-acid levels and the risk of cardiovascular diseases, considering it as a contributing factor to hypertensive diseases [11]. In addition, elevated uric-acid levels are associated with increased cardiovascular morbidity and mortality [47].

4.3 How can we elucidate the impact of Lnt on blood lipid levels and oxidant / antioxidant stress biomarkers?

Research investigating natural substances capable of improving lipid profiles for the prevention of cardiovascular diseases holds significant therapeutic importance [31, 39, 48]. However, there has been limited interest in identifying dietary components that could be incorporated into foods to regulate lipid levels and/or oxidant stress biomarkers. Some authors suggest that Ln may help prevent risk factors associated with cardiovascular diseases, and its bioactive compounds may positively influence blood lipid metabolism [31, 39, 48]. The potential improvement in lipid profiles could be attributed to the flavonoids and glycosides present in Lnt leaves [49, 50]. Despite this, the active components of Ln remain understudied. This study revealed a higher content of flavonoids in Ln extract (409.010 µg/g), with hyperoside (207.971 µg/g) identified as the most abundant compound. Additionally, the phenolic profile of Lnt infusion included flavonoid compounds such as (-)-epicatechin, (+)-catechin, and quercitrin (Data not published yet). These findings align with those of Idowu et al. [51], who reported high antioxidant activity and concentrations of flavonoids and flavones in dried Ln leaves. The positive effects of Ln on human lipid profiles and oxidant stress biomarkers may be associated with the richness of its infusion in flavonoids, especially hyperoside and quercitrin (Data not published yet). Park et al. [52] demonstrated the protective effects of hyperoside against oxidant stress, showcasing its various pharmacological activities, including antioxidant, antidiabetic, and cardioprotective effects [53, 54]. Several studies have highlighted the antioxidant activity of compounds such as kaempferol-3-O-glucoside, quercetin, rutin, and phenolic acids (e.g., caffeic and chlorogenic acids), which act as scavengers of free radicals [55-58]. The validation of our hypothesis regarding the protective effects of Ln against oxidant damage suggests that Lnt infusion may serve as a potential safeguard against oxidant stress-related diseases. This implies that Lnt infusion could emerge as a promising new antioxidant ingredient for integration into medical or functional foods. Furthermore, the observed role of Lnt in upregulating LDL-R expression, resulting in reduced serum LDL-C levels, could be viewed as a novel therapeutic strategy to decrease the incidence of cardiovascular events. In summary, our results underscore the potential of Lnt infusion to augment antioxidant properties and modulate blood lipid levels, thus positioning it as a viable candidate for incorporation into food products. Particularly, Lnt infusion holds promise in preventing oxidant stress associated with various diseases, including cardiovascular diseases.

4.4 Possible mechanisms explaining the variation in lipid profile after only 10 days of Lnt consumption

Several mechanisms can be advanced to elucidate the fluctuations in lipid profile observed following just 10 days of Lnt consumption [59]. Firstly, it is conceivable that compounds inherent in Lnt may exhibit a relatively rapid onset of action or possess high bioavailability, thereby leading to observable effects on lipid metabolism within a brief timeframe [59]. Certain bioactive components in herbal teas can be readily absorbed and metabolized, thereby exerting physiological effects relatively expeditiously [59]. Secondly, the human organism is capable of manifesting swift responses to dietary interventions, especially in the realm of lipid metabolism [59]. Alterations in lipid profiles, including levels of LDL-C, HDL-C, and LDL-R, can be modulated by various factors including dietary intake, metabolic processes, and genetic predispositions [59]. The specific constituents present in Lnt may interact with lipid metabolism pathways, leading to observable changes within a condensed duration. Thirdly, individual responses to dietary interventions may manifest considerable heterogeneity owing to variations in metabolism, composition of gut microbiota, baseline lipid profiles, and overall health status [59]. While some participants may exhibit marked alterations in lipid profiles in response to Lnt consumption, others may demonstrate more modest or

inconsequential effects. Fourthly, although the relatively abbreviated duration of the study (i.e., 10 days), substantial shifts in lipid profiles were observed between pre- and post-intervention assessments. This suggests that even transient periods of dietary intervention with Lnt may exert measurable effects on lipid metabolism, highlighting the potential efficacy of this botanical intervention in modulating cardiovascular risk factors. In summary, the observed variability in lipid profiles subsequent to 10 days of Lnt consumption can be attributed to factors such as the pharmacokinetics properties of bioactive compounds, physiological responses to dietary interventions, individual diversity, and the duration of the intervention. However, further research is imperative to elucidate the underlying mechanisms and validate these findings in larger, long-term (e.g., 90 days) clinical trials.

4.5 Limitations and directions for future research

Our RCT presents some limitations that warrant acknowledgment. Chief among these limitations is the absence of dietary surveys, a critical component in studies evaluating the effects of dietary interventions, such as the consumption of Lnt. This omission poses several risks to the integrity and interpretation of our findings [60]. Firstly, without dietary surveys, it becomes challenging to account for potential confounding variables associated with participants' baseline dietary habits [60]. Variations in individual dietary patterns could influence the measured outcomes, including lipid profiles and oxidative stress biomarkers, thereby affecting the interpretation of the intervention's effects [60]. Secondly, dietary surveys provide valuable insights into participants' baseline nutritional status, including macronutrient intake, micronutrient levels, and overall dietary quality [60]. Absent this information, the assessment of whether changes in lipid profiles and oxidative stress biomarkers are exclusively attributable to the intervention or influenced by pre-existing dietary factors becomes difficult [60]. Thirdly, dietary surveys serve as a means to evaluate participants' adherence to the study protocol, including compliance with the Lnt consumption and potential alterations in other dietary habits over the study duration [60]. The absence of such monitoring may introduce ambiguity regarding participants' actual exposure to the intervention and its consequent impact on study outcomes [60]. Fourthly, the absence of dietary surveys compromises the generalizability of our findings to populations with similar dietary patterns or nutritional habits [60]. Understanding the baseline dietary characteristics of participants is crucial for extrapolating the study results to broader populations and formulating recommendations for dietary interventions [60]. Lastly, the absence of data on participants' dietary intake hinders the interpretation of observed changes in lipid profiles and oxidative stress biomarkers in the context of dietary influences [60]. However, in order to distinguish between the effects of the intervention and those resulting from pre-existing dietary factors or changes in dietary habits during the study period, participants were instructed to maintain their usual dietary pattern and physical activity level throughout the study period. Additionally, participants were advised against consuming any herbal recipes or nutritional supplements that could potentially influence metabolic outcomes. These instructions were provided to ensure consistency and minimize potential confounding factors that could affect the interpretation of our results. Additionally, our findings underscore the necessity for replication with larger sample sizes and further research to elucidate the mechanisms of the active components within Ln leaves that contribute to the enhancement of lipid profiles and oxidant status, particularly the upregulation of LDL-R. Strengthening the evidence base on the beneficial effects of Lnt on human lipid status warrants the inclusion of participants with cardiovascular diseases in future studies. To enhance the robustness of the research, more potent studies conducted over an extended treatment period are necessary. Larger clinical trials employing diverse groups, including placebo and patient cohorts with cardiovascular diseases and varying doses, are imperative to provide a comprehensive understanding of Lnt's potential impact. This approach will contribute to a more robust and reliable body of evidence understanding of the potential impact of Lnt and its active components, contributing to a more robust and reliable body of evidence concerning the therapeutic potential of Lnt and its active components.

5 Conclusion

The study provides partial evidence supporting the favorable effects of Lnt consumption over a ten-day period in healthy volunteers, indicating improvements in lipid parameters and antioxidant biomarkers. The observed capacity of Lnt to increase HDL-C and LDL-R levels, coupled with its antioxidant properties, suggests its potential utility in preventing cardiovascular diseases. However, further research involving larger sample sizes and diverse participant cohorts is warranted to validate and extend these findings.

Declaration. We have looked for assistance from artificial intelligence (ie; language model, ChatGPT 3.5) in the correction and improvement of our scientific paper [61, 62].

Source of support: This research received partial funding from the Tunisian Ministry of Higher Education and Scientific Research.

Acknowledgements: The authors gratefully acknowledge the financial support provided by the Tunisian Ministry of Higher Education and Scientific Research and the Department of Occupational Medicine at Farhat Hached University Hospital, Sousse, Tunisia. Special appreciation

is extended to Professor Samir Boukattaya for his invaluable contribution to enhancing the quality of writing in this article. The language model ChatGPT 3.5 [54] was utilized to correct and improve the academic writing of this paper.

Previous submissions: None.

Authors' Contribution: CC and SM contributed to the design and development of the study; CC, HBS, JM, and MM participated in data analysis and interpretation; CC, HBS, SM, JM, and MM contributed to writing the article or provided critical analysis leading to significant changes in the intellectual content; CC, SM, MS, HG, JM, MM, NM, MN, MBF and HBS contributed to final approval of the version submitted after critical review.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

Preprint deposit: Yes (https://www.researchsquare.com/article/rs-28846/v1)

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