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Effects of daily *Laurus nobilis* tea on inflammatory biomarkers and stress/anxiety levels: a randomized controlled pilot study in healthy North African participants

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To examine the anti-inflammatory and anti-stress/anxiety effects of Laurus nobilis tea (Lnt) in healthy humans. A randomized, double-blind, placebo-controlled trial was conducted with 55 healthy individuals. Participants consumed either Lnt (intervention group, IG; n = 30) or placebo tea (control group, CG; n = 25) once daily for 10 days. Blood samples were collected at two time points: one day before (Day1) and one day (Day11) after the study. Plasma hematological parameters were analyzed, and the neutrophil-to-lymphocyte ratio (NLR) calculated. Stress and anxiety were assessed using the perceived stress scale (PSS) and the Spielberger state-trait anxiety inventory (STAI). The groups were matched for demographic and clinical characteristics. Compared to Day1, the IG showed significant reductions in leucocytes, neutrophils, NLR, PSS, and STAI on Day11. Compared to the CG, the IG had lower leucocytes (p = 0.004), neutrophils (p = 0.001), and NLR (p = 0.014). Significant interactive effects were observed. Additionally, PSS and STAI scores were significantly lower in the IG on Day11 (p = 0.001). Lnt infusion may regulate NLR levels and could serve as an anti-inflammatory agent and potential protector against inflammation-related.

Trial registration: Pan African Clinical Trial Registry (PACTR202205585377035; 13/05/2022). The trial was reported in accordance with the CONSORT reporting guidelines.

Keywords Herbal medicine, Inflammation mediators, Psychological tests, Randomized controlled trials as topic

Abbreviations

CG	Control-group
CONSORT	Consolidated standards of reporting trials
ES	Effect size
IG	Intervention-group
Ln	*Laurus nobilis*
Lnt	*Laurus nobilis* tea
NLR	Neutrophil-to-lymphocyte ratio

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OMD	Occupational Medicine Department
O. syriacum	Origanum. syriacum
PSS	Perceived stress scale
SAP	Statistical analysis plan
STAI	State-trait anxiety inventory

Laurus nobilis (Ln), generally recognized as bay, bay laurel, sweet bay, Roman laurel, or simply laurel, is a tree native to Mediterranean states[1,2]. This aromatic shrub, which appertains to the *Lamiaceae* family, is widely applied to aroma numerous plates, mainly in Mediterranean gastronomy[3–6]. Ln has also been applied in traditional medicine meanwhile ancient times for treating certain chronic conditions (e.g.; rheumatism, cardiac diseases, viral infections) and symptoms (e.g.; cough, diarrhea)[3–6]. Additionally, Ln serves as a common digestive secretion stimulant, antibacterial, diaphoretic, and carminative[3–6]. In North Africa, this herbal is common, and in Tunisia, it is called *Rand*[7]. *Rand* is particularly widespread in Tunisia along riversides, in mountainous areas, and on wet cliffs[7]. The *Rand* tree primarily matures in humid/sub-humid bioclimatic areas, mainly in northwestern regions like Kef, Tabarka, Ain Draham, and Cap Bon[7]. Ln has traditionally been applied for both gastronomic and therapeutic aims[3,4,6]. The new herbal and its dried needles have long been applied for gastronomic and nutrition conservation[3,4,6]. Furthermore, conferring to long-established Tunisian medicine, Ln is extensively applied as a medicinal herbal for treating various conditions, particularly migraines and neuropsychiatric disorders[5]. It has been used as a tranquilizer and for digestive disorders[5]. Ln has demonstrated beneficial properties in the terrain of *invitro* studies and in plant medicine, including antibacterial, antifungal, anti-diabetic, antihyperlipidemic, hepatoprotective, and anti-inflammatory effects[5,8–11]. In this background, and affording to Tunisian ritual, Ln is often consumed as a therapeutic infusion made from fresh or dried leaves (e.g.; Ln tea (Lnt))[8]. Most of the knowledge regarding the therapeutic benefits of Ln on human health comes from herbal medicine and in vitro studies[5,8–11]. However, in vivo tests on laboratory animals have demonstrated an anti-inflammatory effect of methanolic, ethanolic, and dichloromethane-methanolic extracts of Ln[8,12,13].

Several studies have shown that the neutrophil-to-lymphocyte ratio (NLR) is a probable indicator of inflammation in cardiac conditions, cancer, Alzheimer's disease, major depressive disorder, schizophrenia during psychotic episodes, and bipolar disorder during manic episodes[14–18]. Moreover, an elevated NLR is a significant predictor of chronic stress in multiple species, including humans[19]. Increased neutrophil counts and decreased lymphocyte counts have been reported in bipolar disorder patients compared to a control-group (CG)[18]. Additionally, a positive correlation has been identified between suicidal behavior in bipolar disorder and NLR[20], and schizophrenia patients have been reported to exhibit high NLR values[21].

To the finest of the authors' awareness, no preceding study has examined the effects of Lnt infusion on inflammatory parameters and stress/anxiety biomarkers. The purpose of this randomized, double-blind, controlled trial including two groups of individuals (intervention-group (IG) vs. CG) was to examine the effects of Lnt consumption on various complete blood count components, including NLR, as well as on stress and anxiety scales. The null postulate was that both groups would have a similar inflammation status (i.e.; comparable NLR values) 10 days after the intervention.

Population and methods

This study is a fragment of a larger project consisting of two components. The first component, published in 2024[22], targeted to examine the effects of Lnt consumption on lipid data and oxidative/antioxidative stress biomarkers in healthy humans. The second component is the focus of the current study. For this reason, a significant portion of the methodology has already been described in a previous publication[22].

Study design

This was a randomized, double-blind, controlled trial conducted over one month (from May 4 to May 31, 2022). It was carried out in the occupational medicine department (OMD) and the biochemistry laboratory at Farhat Hached Hospital (Sousse, Tunisia).

All methods were performed in accordance with the relevant guidelines and regulations, including the ethical standards of the institutional research committee and the principles of the Declaration of Helsinki. The study was conducted following the guidelines established by the consolidated standards of reporting trials (CONSORT) statement[23]. Study approval was got from the medical research and ethics committee (Faculty of Medicine of Sousse, Approval No. CEFMS 17/2019). Written informed consent was acquired from all individuals. All tests were changeless, and individuals obtained a report of their results. The study was recorded in the Pan African Clinical Trial Registry database (www.pactr.org; PACTR202205585377035) on May 13, 2022.

Patient and public involvement

Patients and members of the public were not involved in defining the research question, designing the methodology, collecting the data, analyzing or interpreting the results, or preparing the manuscript.

Changes to trial protocol

No changes to the protocol were made after the trial commenced.

Sample size

The null postulate[24] was H_0: $m_1 = m_2$ and the alternate postulate was H_a: $m_1 = m_2 + d$, where "*d*" is the difference between two means, and n_1 and n_2 are the sample sizes for the IG and the CG, such $N = n_1 + n_2$.

The sample size was appraised using this formulation[24]: $N = [(r+1)\,(Z_{1-\alpha/2} + Z_{1-\beta})^2\,\delta^2]/r\,d^2$, where.

- "$Z_{1-\alpha/2}$" is the normal deviate at a level of significance ($Z_{1-\alpha/2}$ = 1.28 for 20% level of significance);
- "$Z_{1-\beta}$" is the normal deviate at 1-β% power with β% of type II error ($Z_{1-\beta}$ = 0.52 at 70% statistical power);
- "r" (= n_1/n_2) is the ratio of sample size necessary for the two groups (r = 1 gives the sample size distribution as 1:1 for two groups);
- "s" and "d" are the pooled standard-deviation and difference of NLR means of the two groups. Given the innovator design of this study, the authors arbitrarily fixed the latter two values at 1.70 and 2.50, for the IG and the CG, respectively, with a common standard-deviation equal to 2.22.

The sample size for the study was 50 individuals (25 in each group). Assumption of 20% for absence through the second visit and non-drinking of Lnt/placebo tea gave a revised total sample of 62 individuals (62 = 50/(1-0.20)).

Recruitment and study population

Participant recruitment took place between May 4 and May 31, 2022. Follow-up ended on Day_{11} for each participant, corresponding to 10 consecutive days of intervention. The total duration of follow-up for all outcomes was therefore 11 days. The trial was completed as planned and was not stopped early.

Individuals were recruited through three methods: (i) Residents, medical staff members of the aforementioned OMD and interns (n = 62) were requested by a senior author (*MM* in the authors' list); (ii) Residents and interns of the above-mentioned biochemistry laboratory (n = 55) were invited by a senior author (*SM* in the authors' list) to participate in the study; and (iii) An announcement was made within the aforementioned OMD to invite healthy individuals accompanying their patients to take part in the study.

The following inclusion criteria were applied: Non-smokers, non-vegetarians, not pregnant, no use of medication or nutritional supplements, no chronic diseases (e.g.; diabetes mellitus, arterial hypertension, psychiatric, cardiovascular diseases, gastrointestinal problems, asthma) and unknown diet allergies.

Exclusion criteria included absence during the second visit (i.e.; Day_{11} of the study) or failure to consume Lnt or placebo tea one or more times during the 10-day study period. No eligibility criteria were applied to study sites or to individuals delivering the intervention, as the trial did not involve specialized personnel or procedures requiring specific training.

Random allocation and blinding

Individuals were randomly allocated to one of two groups: the IG (i.e.; Lnt once a day for 10 successive days) or the CG (i.e.; placebo tea once a day for 10 successive days). A predefined sequence, generated using Random Allocation software, version 1.0.0 (M. Saghaei, Isfahan University of Medical Sciences, Isfahan, Iran), was used. Individuals were enrolled in the study according to the order of recruitment.

Both individuals and investigators were unbending to group division. The allocation arrangement was hidden by a physician not involved in the study and was only revealed to the investigators after all assessments had been completed.

Practical procedure and examinations achieved

Figure 1 illustrates the study procedure, which includes two appointment conducted 10 days apart. During the first visit (Day_1), a medical questionnaire was administered, weight was measured, a physical examination was performed, a blood sample was collected, and each individual received the required amount of tea (i.e.; Lnt for the IG and placebo for the CG). During the second visit (Day_{11}), which took place 10 days after the consumption of Lnt or placebo tea, individuals were asked about their tolerability to Lnt consumption, a medical questionnaire was administered, and a blood sample was collected.

Medical questionnaire

The medical questionnaire consisted of three parts. The first gathered demographic information about the individuals (e.g.; sex, age, weight). The second and third parts assessed the perceived stress scale (PSS)[25], and the Spielberger state-trait anxiety inventory (STAI)[26], respectively. All individuals completed the PSS and STAI twice: on Day_1 and Day_{11} (i.e.; after 10 consecutive days of Lnt or placebo infusion consumption). Stress was measured by the validated Arabic sort of the PSS questionnaire[27]. The latter is one of the most widely used tools for measuring psychological stress[25]. It was designed to evaluate the degree to which individuals observe their lives as impulsive, overwhelming over the previous month and uncontrollable. The 14 items were evaluated on a five-point occurrence scale, fluctuating from 0 "never" to 4 "very often" within the previous month. The entire PSS score was got by reversing the recording of positive items and making the scores of entirely 14 items.

The STAI measures feelings of apprehension, tension, nervousness, and anxiety in response to anxiety-provoking situations or competitive settings[26]. It is a 20-item self-report amount of anxiety, by a 4-point Likert-type scale (ranging from 0 to 4 points) for each item. The state scale contains 10 reverse-scored items, while the trait scale consists of 10 direct-scored items. For this study, the French version of the STAI[28] was consulted, and the questions were translated into Arabic. The translation process involved a discussion between the researcher conducting the survey (*CC* in the authors' list) and the study leader (*BSH* in the authors' list) to clarify specific questions and standardize their phrasing. Additionally, a role-play exercise was conducted, exploring various scenarios for answering the questions to ensure consistency in data collection.

Blood data

Leucocytes (normal range: 4–11 $10^3/mm^3$), neutrophils (normal range: 2.0–7.7 $10^3/mm^3$), lymphocytes (normal range: 1.20–4.95 $10^3/mm^3$), platelets (normal range: 150–400 $10^3/mm^3$) and monocytes (normal range: 0.10–0.66 $10^3/mm^3$), eosinophils (normal range: 0.1–0.5 $10^3/mm^3$), Hemoglobin (g/dL) (normal ranges: Women: 12–16 g/dL; men: 14–18 g/dL) and Hematocrit (%) (normal range: Women: 37%–47%; Men: 42%–50%)[29] were

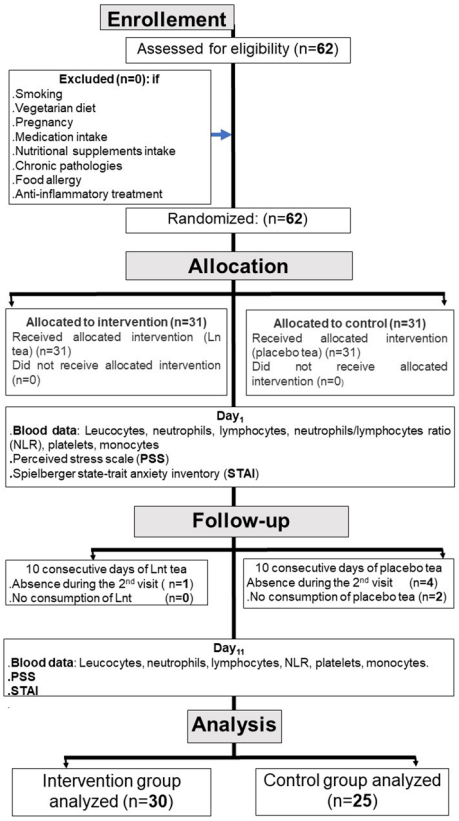


Fig. 1. CONSORT flowchart of the recruitment process for individuals in the trial. *Ln Laurus nobilis*. *n* number. *NLR* Neutrophil-to-lymphocyte ratio. *PSS* Perceived stress scale. *STAI* State-trait anxiety inventory.

measured from a 4 mL whole blood sample using automated counters. Inflammation status was assessed using the NLR, which was calculated manually.

Herbal material

Individuals were instructed to consume either Lnt (i.e.; the IG) or placebo tea (i.e.; the CG) daily at 7 p.m. for 10 successive days. They were also advised to keep their ordinary daily routine, including food intake and physical activity, throughout the study period.

For the IG, individuals prepared Lnt by steeping five grams of dried Lnt leaves in 100 mL of boiling water for 15 min, then straining the infusion. The daily Lnt dose was standardized at five grams (approximately the weight of two tablespoons) aligning with the plant's traditional use. Consistent with one previous study[10], a 10-day study period is considered methodologically valid. The Ln leaves were gathered between March and May 2021 from Hammam Jedidi-Mount Mount, Zaghouan, a sub-humid region in Tunisia. A herbarium specialist at the higher school of agriculture of Mograne, Carthage university, identified the plant material using the flora of Tunisia[7].

The placebo material was derived from the hard stems of *Origanum syriacum L (O. syriacum)*[30]. *O. syriacum* stems were selected because, unlike the leaves and flowering parts traditionally used for medicinal purposes, the lignified stems contain lower concentrations of bioactive constituents and, after the depletion procedure, provide a plant-based infusion with sensory characteristics comparable to the intervention tea while minimizing exposure to phytochemicals.

The stems were thoroughly cleaned and boiled for five hours, with the water replaced every hour. This prolonged boiling process aimed to reduce the content of water-soluble components[30]. Five grams of the placebo material were used to prepare the infusion with 100 mL of boiled water. The leaves were steeped for 15 min and then filtered.

To verify the effectiveness of the depletion procedure, aqueous extracts of *O. syriacum* stem material were prepared before and after boiling and analyzed using HPLC-ESI-MS and HPLC-DAD. The chromatographic profiles and concentrations of selected bioactive compounds were compared to assess the reduction of water-soluble phytochemicals in the placebo material.

In both trial groups, no relevant concomitant care or treatments were reported. Participants were instructed to maintain their usual daily routines, including diet and physical activity, and to avoid taking any medications or nutritional supplements during the 10-day intervention period. Based on the medical questionnaires administered on Day_1 and Day_{11}, no participant received concomitant interventions that could have influenced the study outcomes.

Preparation of Ln leaf aqueous extract and analysis of bioactive compounds

Fresh Ln leaves were kept at ambient temperature, protected from sunshine, and stored in an airy room until completely dry. Five grams of dried Ln leaves were utilized to prepare the infusion with 100 mL of hot water. The leaves were steeped for 15 min and then filtered. The infusion was kept at 20 °C for one month before chromatographic analysis.

The infusion extract was analyzed by high-performance liquid chromatography combined with electrospray ionization mass spectrometry operating in both negative and positive ionization modes, as well as by high-performance liquid chromatography with a diode-array detector, to characterize and quantify bioactive compounds.

The same analytical procedure was applied to *O. syriacum* stem extracts before and after the boiling treatment used for placebo preparation in order to verify the effectiveness of the depletion procedure in reducing water-soluble phytochemicals.

Eighteen out of the 30 monitored compounds were identified in the analyzed Lnt extracts. Box 1 presents the content, expressed as µg/g of dried weight extract, of the 30 monitored compounds quantified in the *Ln* extract. The extract contained a total of 417.473 µg/g of monitored bioactive compounds. Flavonoids were the most abundant class of bioactive compounds (409.010 µg/g), with *quercitrin* (89.742 µg/g), *hyperoside* (207.971 µg/g) and *(-)-Epicatechin* (50.614 µg/g) being the most concentrated molecules.

Statistical analysis

The Kolmogorov-Smirnov test confirmed that the quantitative data followed a normal distribution. Consequently, the results were presented as mean±standard-deviation (95% confidence interval). Sex was expressed as number (%). Changes in the evaluated data were calculated as ΔData (= Day_{11} value minus Day_1 value). The statistical analysis plan (SAP) was defined a priori, before data collection and before unblinding, in accordance with CONSORT recommendations[23]. No adjustment for multiplicity was planned or conducted, as predefined in the SAP. No interim analyses or stopping rules were planned. Missing data were excluded from analyses and no imputation procedures were applied. All analyses were conducted on the per protocol population, as defined a priori.

Multiple comparisons were conducted. A two-sided chi-square test was conducted to compare categorical data (e.g.; sex) between the two groups. A Student t-test was conducted to compare: (i) Data from Day_1 and Day_{11} across each group, (ii) Data of the IG and the CG on the same day, and (iii) ΔData between the two groups. Hedges' unbiased d values were used to assess effect size (ES), interpreted as follows: values of ≤ 0.2 indicated a minor ES, approximately 0.5 represented a moderate ES, around 0.8 signified a large ES, and values exceeding 1.30 denoted a very large ES[31]. To examine the highest-order interactions among multiple categorical independent variables (i.e.; comparison between two groups (IG vs. CG) and two time points (Day_1 vs. Day_{11}), a factorial analysis of variance was conducted. ES were calculated as partial eta-squared to assess the potential practical significance of the findings. A large ES was determined as ≥ 0.25, medium as > 0.09 and small as ≤ 0.09[32].

All statistical procedures were performed using STATISTICA (data analysis software system, version 12. www.statsoft.com, RRID: SCR_014213). For all tests, the significance level was established at $p < 0.05$.

No.	Compound	Content (µg/g of dried extract)
1	Shikimic acid	0.409
2	Gallic acid	0.071
3	3-Caffeoylquinic acid	1.211
4	(+)-Catechin	19.223
5	5-Caffeoylquinic acid	1.549
6	Caffeic acid	0.681
7	(-)-Epicatechin	50.614
8	Syringic acid	0.451
9	p-Coumaric acid	0.561
10	Ferulic acid	1.354
11	3,5-Dicaffeoylquinic acid	0.948
12	Naringin	0.196
13	Rutin	10.547
14	Hyperoside	207.971
15	Trans-cinnamic acid	1.226
16	Kaempferol-3-glucoside	25.902
17	Quercitrin	89.742
18	Quercetin	4.815
Total phenolic compounds		8.053
Total flavonoid compounds		409.010
Total compounds		417.473

Box 1. Content of different bioactive compounds found in *Laurus nobilis* extracts.

Results

Sixty-two individuals were enrolled in the study. Data from seven individuals (including one from the IG) were removed from the final statistical analysis. As a result, 55 individuals completed the study (30 in the IG) (Fig. 1).

The two groups were balanced in terms of sex, age, and weight (Table 1). During the 10-day period of Lnt infusion consumption, no significant events that could have affected the study protocol were observed. On Day_1, the two groups had comparable hematological data as well as PSS and STAI scores (Table 1).

Validation of the placebo preparation

To validate the placebo preparation, the phytochemical composition of *O. syriacum* stem extracts was determined before and after the depletion procedure. As shown in Box 2, prolonged boiling with hourly water replacement markedly reduced the concentration of detectable bioactive compounds. Total quantified compounds decreased from 60.224 to 6.012 µg/g dry extract, corresponding to a 90.0% reduction. Total phenolic compounds and flavonoids decreased from 50.376 to 4.862 µg/g and from 9.848 to 1.150 µg/g, corresponding to reductions of 90.4% and 88.3%, respectively. In addition, no major flavonoids identified in the Lnt infusion, including hyperoside, quercitrin and (-)-epicatechin, were detected at appreciable concentrations in the placebo after the depletion procedure.

Effect of Lnt consumption on hematological parameters

Hematological data for both groups is presented in Table 2. The key findings were as follows:

(i) In the IG, compared to Day_1, leucocytes, neutrophil, and NLR values were significantly decreased on Day_{11} by 1.02, 1.17, and 0.62, respectively. In the CG, no statistically significant changes were observed.
(ii) On Day_{11}, compared to the CG, the IG exhibited lower leucocytes, neutrophil, and NLR values. The ES for NLR was small with Hedges' unbiased d = −0.410.
(iii) Significant interactive effects were detected between the two groups (IG vs. CG) and the two time points (Day_1 vs. Day_{11}) for leucocytes, neutrophils, and NLR. The ESs for all these parameters were medium.

Impact of Lnt consumption on PSS and STAI scores

Table 3 presents the PSS and STAI score data for both groups. The key findings were as follows:

(i) In the IG, compared to Day_1, PSS and STAI scores were significantly reduced on Day_{11} by 8.39 and 11.14, respectively. In the CG, no statistically significant changes were observed.
(ii) On Day_{11}, compared to the CG, the IG exhibited lower PSS and STAI scores.
(iii) Significant interactive effects were observed between the two groups (IG vs. CG) and the two time points (Day_1 vs. Day_{11}) for PSS and STAI scores. The ES medium for PSS and small for STAI.

Discussion

Lnt consumption had significant effects on inflammatory biomarkers (i.e.; leucocytes, neutrophils, and NLR) as well as PSS and STAI scores. Therefore, our null hypothesis (i.e.; that the two groups would have comparable NLR values 10 days after the intervention) was rejected. As far as the authors are aware, this is the first randomized,

Data	Unit	Intervention-group (n=30)	Control-group (n=25)	p-value	Effect size
Sex and anthropometric data					
Sex	Women	26 (86.66)	20 (80.00)	0.506	-
Age	Year	37±9 (34 to 41)	36±9 (33 to 40)	0.643	0.11[a]
Weight	kg	68±9 (65 to 75)	70±12 (65 to 72)	0.573	-0.18[a]
Initial hematological data					
Leucocytes	$10^3/mm^3$	6.00±1.34 (5.50 to 6.50)	5.74±1.08 (5.29 to 6.18)	0.436	+0.21[b]
Neutrophils	$10^3/mm^3$	3.52±1.05 (3.12 to 3.91)	3.62±0.90 (3.25 to 3.99)	0.689	-0.10[a]
Lymphocytes	$10^3/mm^3$	1.93±0.47 (1.76 to 2.11)	2.59±2.07 (1.73 to 3.44)	0.098	-0.45[b]
Neutrophils/lymphocytes ratio	absolute	1.92±0.73 (1.64 to 2.19)	1.88±0.96 (1.49 to 2.28)	0.880	+0.05[a]
Monocytes	$10^3/mm^3$	7.40±1.22 (6.95 to 7.86)	7.40±1.47 (6.79 to 8.01)	0.993	0.00[a]
Eosinophils	$10^3/mm^3$	2.29±1.74 (1.64 to 2.94)	2.24±1.54 (1.61 to 2.88)	0.914	0.03[a]
Platelets	$10^3/mm^3$	239.80±59.91 (217.43 to 262.17)	240.04±63.06 (214.01 to 266.07)	0.988	-0.01[a]
Hemoglobin	g/dL	13.56±1.31 (13.07 to 14.05)	13.30±1.19 (12.81 to 13.79)	0.455	+0.20[a]
Hematocrit	%	40.04±3.37 (38.76 to 41.33)	40.22±3.59 (38.74 to 41.70)	0.858	-0.05[c]
Initial perceived stress scale and Spielberger state-trait anxiety inventory (STAI) scores					
Perceived stress scale	Absolute	29.17±7.36 (26.37 to 31.97)	29.12±6.84 (26.30 to 31.94)	0.979	+0.01[a]
STAI	Absolute	48.57±10.71 (44.7 to 52.57)	50.32±10.97 (45.79 to 54.85)	0.552	-0.16[a]

Table 1. Characteristics of healthy volunteers divided into two groups. Quantitative data were mean± standard deviation (95% confidence interval), and sex was number (%). p-values: Student t test or 2-sided chi-2 test between the 2 groups. Effect size (Hedges' unbiased d): [a]Minor, [b]Moderate, [c]Large, [d]Very large.

No.	Compound	Content (µg/g) in *Origanum syriacum L*extract	Content (µg/g) in *Origanum syriacum L*extract after boiling
1	Shikimic acid	0.112	*n.d.
2	Gallic acid	0.312	0.10
3	Loganic acid	*n.d.	*n.d.
4	3-Caffeoylquinic acid	0.552	0.012
5	Swertiamarin	*n.d.	*n.d.
6	Gentiopicroside	*n.d.	*n.d.
7	(+)-Catechin	*n.d.	*n.d.
8	Delphinidin-3,5-diglucoside	*n.d.	*n.d.
9	Sweroside	*n.d.	*n.d.
10	5-Caffeoylquinic acid	6.823	0.13
11	Caffeine	*n.d.	*n.d.
12	Cyanidin-3-glucoside	2.331	0.54
13	Vanillic acid	*n.d.	*n.d.
14	Caffeic acid	32.241	2.13
15	(-)-Epicatechin	*n.d.	*n.d.
16	Syringic acid	1.431	0.21
17	*p*-Coumaric acid	2.610	0.18
18	Ferulic acid	1.123	*n.d.
19	3,5-Dicaffeoylquinic acid	4.321	2.1
20	Quinine	*n.d.	*n.d.
21	Naringin	*n.d.	*n.d.
22	Rutin	0.234	*n.d.
23	Hyperoside	4.123	0.51
24	*Trans*-cinnamic acid	0.851	*n.d.
25	Resveratrol	*n.d.	*n.d.
26	Amarogentin	*n.d.	*n.d.
27	Kaempferol-3-glucoside	0.713	*n.d.
28	Quercitrin	0.311	*n.d.
29	Quercetin	2.136	0.10
30	Isogentisin	*n.d.	*n.d.
Total phenolic compounds		50.376	4.862
Total flavonoid compounds		9.848	1.150
Total compounds		60.224	6.012

Box 2. Content (µg/g dry extract) of bioactive compounds in *Origanum syriacum L* extracts before and after boiling. * n.d., not detectable; Relative Standard Deviation (RSD %) was 2.7–8.6% for all compounds

double-blind, placebo-controlled clinical trial examining the effects of Lnt infusion on inflammatory biomarkers, PSS, and STAI scores.

A major challenge in herbal intervention trials is the selection of an appropriate placebo because plant-derived materials may contain biologically active constituents. In the present study, the placebo was prepared from *O. syriacum* stems subjected to prolonged boiling with repeated water replacement. HPLC-ESI-MS and HPLC-DAD analyses demonstrated that the depletion procedure reduced total quantified phytochemicals from 60.224 to 6.012 µg/g dry extract (90.0% reduction). The residual phytochemical content represented only 1.4% of that measured in the Lnt infusion (417.473 µg/g). These findings support the use of the treated *O. syriacum* stem material as a low-phytochemical placebo designed to preserve sensory characteristics while minimizing potential biological activity. Nevertheless, as with most plant-derived placebos, the complete absence of residual bioactive compounds cannot be entirely excluded.

Analysis of the Interactions between Lnt infusion, stress, NLR, and metabolic markers

The primary finding of this study was that Lnt consumption in healthy individuals led to a decrease in NLR, leucocytes, and neutrophil levels. However, the anti-inflammatory activity of Lnt infusion has not been evaluated until now. While several studies have examined the anti-inflammatory effects of Lnt in organic extracts or essential oils, its impact as an infusion remains unexplored[5,33,34]. The interactions between the central nervous system, stress, and the immune system are well documented with a particular focus on the role of cytokines in cell signaling and their function as mediators between these systems. Stress is associated with the stimulation of several neurohormonal pathways, including the autonomic nervous system (specifically the sympathetic division) and the hypothalamic-pituitary-adrenal axis[35]. Stress can lead to increased norepinephrine and epinephrine levels, which may alter lymphocyte, monocyte, and leukocyte function[35]. NLR is an important indicator of inflammation and serves as a simple, cost-effective, and practical marker that reflects the intensity

Data (unit)	Groups	Day_1	Day_{11}	ΔData	p-value: Day_1 vs. Day_{11}	Factorial ANOVA	ES: Partial eta-squared
Leucocytes (10^3/mm^3)	IG	6.00±1.34	4.98±1.27	-1.01±0.64	0.004*	F(1.106)=7.045 p=0.009¥	0.0623[b]
	CG	5.74±1.08	5.93±0.98	0.19±0.69	0.507		
	p-value: IG vs. CG	0.435	0.004*	0.001*	–	–	–
	ES: Hedges' unbiased d	+0.20[a]	-0.82[c]	–	--	–	–
Neutrophils (10^3/mm^3)	IG	3.52±1.05	2.35±0.98	-1.2±0.52	0.001*	F(1.106)=12.551 p=0.001¥	0.1059[b]
	CG	3.62±0.90	3.76±0.90	0.14±0.85	0.5852		
	p-value: IG vs. CG	0.689	0.001*	0.001*	–	–	–
	ES: Hedges' unbiased d	-0.10[a]	-1.47[c]	–	–	–	–
Lymphocytes (10^3/mm^3)	IG	1.93±0.47	2.03±0.58	0.12±0.43	0.494	F(1.104)=0.113 p=0.738	0.0011[a]
	CG	2.59±2.07	2.50±1.84	-0.08±0.51	0.531		
	p-value: IG vs. CG	0.098	0.199	0.115	–	–	–
	ES: Hedges' unbiased d	-0.45[b]	-0.35[b]	–	–	–	–
NLR (Absolute)	IG	1.92±0.73	1.30±0.78	-0.62±0.42	0.003*	F(1.104)=5.065 p=0.027¥	0.0464[b]
	CG	1.88±0.96	1.83±0.74	-0.05±0.57	0.8001		
	p-value: IG vs. CG	0.880	0.015*	0.001*	–	–	–
	ES: Hedges' unbiased d	+0.05[a]	-0.69[c]	–	–	–	–
Monocytes (10^3/mm^3)	IG	7.40±1.22	7.66±1.55	0.26±1.06	0.474	F(1.106)=0.109 p=0.742	0.0010[a]
	CG	7.40±1.47	7.48±1.44	0.08±0.43	0.846		
	p-value: IG vs. CG	0.992	0.654	0.429	–	–	–
	ES: Hedges' unbiased d	0.00[a]	+0.12[a]	–	–	–	–
Eosinophils (10^3/mm^3)	IG	2.29±1.74	2.52±2.04	0.23±1.10	0.645	F(1.103)=0.049 p=0.825	0.0005[a]
	CG	2.24±1.54	2.36±1.72	0.12±1.35	0.712		
	p-value: IG vs. CG	0.914	0.768	0.754	–	–	–
	ES: Hedges' unbiased d	+0.03[a]	+0.08[a]	–	–	–	–
Platelets (10^3/mm^3)	IG	239.80±59.91	245.17±61.86	5.37±18.6	0.734	F(1.106)=0.061 p=0.804	0.0006[a]
	CG	240.04±63.06	239.52±62.71	-0.52±3.89	0.977		
	p-value: IG vs. CG	0.988	0.739	0.127	–	–	–
	ES: Hedges' unbiased d	-0.04[a]	+0.09[a]	–	–	–	–
Hemoglobin (g/dL)	IG	13.56±1.31	13.44±1.36	-0.12±0.36	0.737	F(1.106)=0.1315 p=0.718	0.0012[a]
	CG	13.30±1.19	13.36±1.32	0.06±1.54	0.8578		
	p-value: IG vs. CG	0.455	0.835	0.535	–		
	ES: Hedges' unbiased d	+0.20[a]	+0.06[a]	–	–	–	
Hematocrit (%)	IG	40.04±3.37	39.63±3.80	-0.26±1.25	0.659	F(1.105)=0.004 p=0.945	0.0001[a]
	CG	40.22±3.59	39.89±3.43	-0.32±0.74	0.748		
	p-value: IG vs. CG	0.857	0.789	0.819	–	–	
	ES: Hedges' unbiased d	-0.05[a]	-0.07[a]	–	–	–	

Table 2. Hematological data of the two groups: Interventional-group (IG, n=30) and control-group (CG, n=25). ANOVA: Analysis of variance. ES: Effect size. NLR: neutrophils/lymphocytes ratio. 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. ES (Hedges' unbiased d): [a]Minor, [b]Moderate, [c]Large, [d]Very large. ES (partial eta-squared): [a]Small, [b]Medium, [c]Large.

of stress or systemic inflammation[36–39]. Some studies have reported higher NLR values in psychiatric and neurological conditions such as, depression, schizophrenia and bipolar disorder[16,18,36,39]. Additionally, NLR has been used as a biomarker in conditions such as obesity, diabetes mellitus, coronary artery disease, and arterial hypertension[40–44]. Given its broad clinical relevance, NLR is also linked to physical and metabolic parameters, including body mass index, body weight,, fasting blood sugar, serum lipid concentrations and blood pressure[45].

Anti-inflammatory properties of Ln and comparison with conventional therapies

Since natural medicines are generally associated with fewer side effects, Ln is often used as an alternative therapy[34]. Studies have demonstrated that Ln possesses anti-inflammatory, analgesic, antimicrobial, antioxidant, and anticonvulsant properties[11,18,34,46,47]. Some research has reported that Ln exhibits anti-inflammatory properties similar to standard analgesics and non-steroidal anti-inflammatory drugs like aspirin[48] or dexamethasone[49]. Various Ln leaf extracts have also shown anti-inflammatory effects[50]. Dichloromethane-methanolic extract regulates nuclear factor-kappa B [50], Methanolic extract inhibits nitric oxide production[8], Dimethyl sulfoxide extract reduces the expression of cyclooxygenase-2 and decreases levels of pro-inflammatory interleukin-6 [51], ethanolic extract has also been tested for its anti-inflammatory activity[13].

Data (unit)	Groups	Day_1	Day_{11}	ΔData	p-value: Day_1 vs. Day_{11}	Factorial ANOVA	ES: Partial eta-squared
PSS (absolute)	IG	29.17±7.36	20.78±6.19	-8±8.53	10^{-4*}	F(1.102)=11.258, p=0.0011¥	0.0994[b]
	CG	29.12±6.84	29.60±6.69	0.48±1.36	0.8030		
	p-value: IG vs. CG	0.9786	10^{-4*}	10^{-4*}	–	–	–
	ES: Hedges' unbiased d	0.001[a]	+1.344	–	–	–	–
STAI (absolute)	IG	48.57±10.71	37.43±7.12	-11±7.84	10^{-4*}	F(1.106)=9.4381, p=0.0027¥ –	0.0816[a]
	CG	50.32±10.97	50.76±10.35	0.44±1.58	0.8846		
	p-value: IG vs. CG	0.5525	10^{-4*}	10^{-4*}	–	–	–
	ES: Hedges' unbiased d	+0.159[a]	+1.455	–	–	–	–

Table 3. Clinical assessments of perceived stress scale (PSS) and Spielberger state-trait anxiety inventory (STAI) scores of the 2 groups: intervention-group (IG, n=30) and control-group (CG, n=25). 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. ES (Hedges' unbiased d): [a]Minor, [b]Moderate, [c]Large, [d]Very large. ES (partial eta-squared): [a]Small, [b]Medium, [c]Large.

Associations between NLR, depressive and anxiety scales, and hematological parameters in healthy individuals

In this study, we identified a positive correlation between NLR and depressive and anxiety scales. NLR was significantly correlated with these scales, which are commonly used to assess depression and anxiety in healthy individuals prior to tea consumption[18,51]. Our findings align with previous studies, suggesting that NLR levels increase as a key indicator of inflammation in healthy individuals experiencing stress[18,52]. To the finest of the authors' knowledge, no published study has directly compared depressive and anxiety scales with NLR in the existing literature.

How can we explain the effects of Lnt on blood inflammation biomarkers and clinical assessment scores?

Similar to our approach, some researchers have demonstrated that anxiety can be prevented or treated through a diet rich in phytochemical secondary metabolites, such as polyphenols, including flavonoids and phenolic acids[53]. Natarajan et al.[54] reported that a combination of different polyphenols (e.g.; gallate, catechin, tannic acid, and quercetin) offers protective effects against cartilage degradation while also reducing inflammation. Yoon et al.[55] reported that *kaempferol* may serve as an anti-inflammatory agent by suppressing inflammatory cytokines. Additionally, *p-coumaric acid*, a polyphenol with potent immunosuppressive activity, was detected in Ln extracts[56]. This compound significantly reduced the expression of tumor necrosis factor-α in adjuvant-induced arthritic rats[56].

The primary active phenolic compounds in Ln infusion are flavonoids, with hyperoside being particularly abundant (i.e.; 207.971 µg/g of dry extract). Hyperoside possesses a range of pharmacological effects, including anti-inflammatory, antioxidant, antidiabetic, anticancer, cardioprotective, and antithrombotic properties in various experimental models[57–64]. LnT also has a maximal inhibitory effect on tumor necrosis factor-α, interleukin-6, and nitric oxide[57]. Ku et al.[59] demonstrated that hyperoside is a promising therapeutic agent for vascular inflammatory diseases. In this context, *sesquiterpenes* and flavonoids in the methanolic extract of Ln are believed to play a key role in inhibiting nitric oxide generation[8,12,13]. Furthermore, *spirafolide* and *costunolide*, both present in Ln, have been recognized as potential compounds for the treatment and prevention of various diseases[8,65]. Given its rich flavonoid content, Lnt infusion may be a valuable therapeutic agent for cardiovascular disease treatment.

Study limitations

This study has at least two limitations. Firstly, the assessment of the anti-inflammatory effects of Lnt infusion was based solely on NLR. As suggested by some authors[35,37,39], it would have been more comprehensive to consider additional inflammatory markers and analyze other cytokines in conjunction with NLR to better assess the anti-inflammatory effects of Lnt infusion. However, NLR is a widely recognized inflammation marker and is a simple, cost-effective, practical, and reliable method for assessing stress intensity and systemic inflammation[35,37,39]. Second, investigating the potential benefits of Lnt on lipid metabolism would have been more robust if the study had included a group diagnosed with cardiovascular disease and a placebo group over a longer study period. However, Khan et al. [10] reported that consuming bay leaves for 10 days can cause changes in serum lipid levels. The study found that these levels remained low even after participants discontinued consumption Ln for 10 days, suggesting that daily consumption of Ln infusion may not be necessary. These findings support the validity of a 10-day study period from a methodological standpoint.

Conclusion

NLR tends to be higher in individuals experiencing stress and anxiety but is significantly reduced after consuming Lnt. Therefore, Lnt infusion could be considered a potential anti-inflammatory agent and a protective measure against certain inflammation-related diseases.

Data availability

All data related to this research are presented within the text, tables, and figures. De-identified individual participant data, the data dictionary, and the statistical code will be available upon justified request to the corresponding author and subject to committee approval. Materials related to the intervention can also be provided upon request, pending approval. Data will be shared after manuscript acceptance and upon signing a data-use agreement, in accordance with CONSORT recommendations[23].

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Author contributions

CC, MM and HS are responsible for design and development of the study; CC, MM, HS and BSH contributed to data analysis and interpretation; CC, MM, HS and BSH contributed to writing the article or critical analysis leading to significant changes to the intellectual content; CC, MM, HS, SA, GC, FM, SH, BSC, BBHJ, MBF and BSH contributed to final approval of the version submitted after critical review.

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Declarations

Competing interests
The authors declare no competing interests[66].

Additional information
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