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Nasturtium (*Tropaeolum majus L.*) sub-chronic consumption modulates oxidative stress biomarker response in prediabetic subjects: A pilot study

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ABSTRACT

Background: Nasturtium (*Tropaeolum majus L*.), an edible flower originating from the Andean region of South America, is rich in phenolic compounds derived from quinic acid, flavonoids, glucosinolates (GLS), and their secondary metabolites, isothiocyanates (ITCs), which possess antioxidant and anti-inflammatory effects.

Objective: This study evaluated the impact of sub-chronic consumption of a freeze-dried nasturtium leaves drink on inflammatory and antioxidant biomarkers in individuals diagnosed with prediabetes.

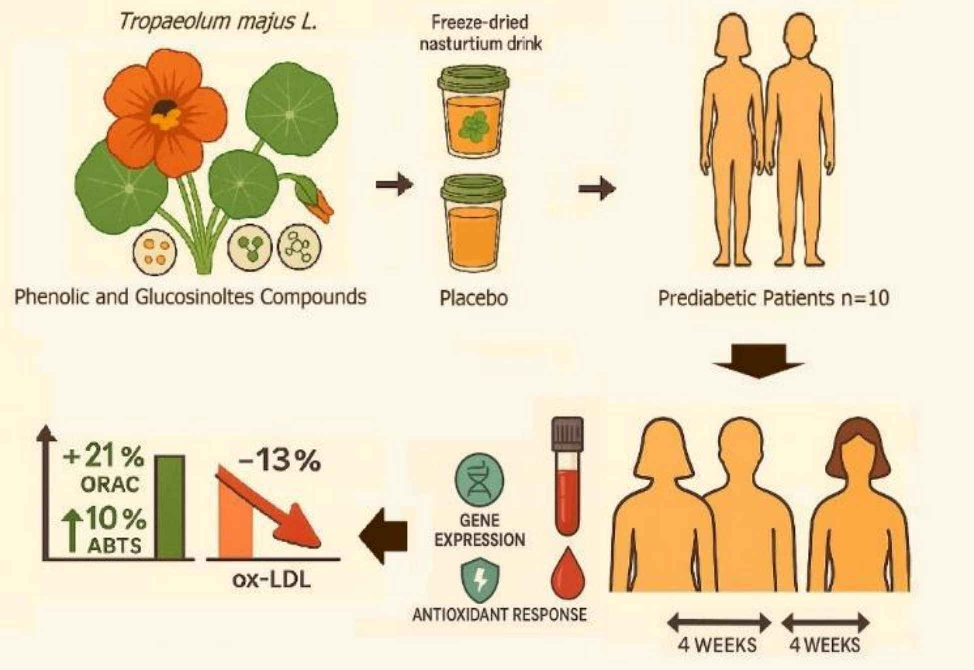
Methods: In this randomized, crossover trial, 10 prediabetic adults (aged 25-70 years) received either nasturtium (NT) or placebo (PLC) for 4 weeks, then crossed over to the other treatment for another 4 weeks. Blood samples before and after each treatment were analyzed for gene expression related to inflammation and antioxidant response, and total antioxidant capacity (TAC) was measured using ORAC and ABTS assays, along with oxidized LDL (ox-LDL) levels.

Results: Weekly consumption of 15 g NT for four weeks significantly increased TAC by 21% (ORAC) and 10% (ABTS), and reduced ox-LDL by 13%. No changes in gene expression were observed.

Novelty of the Study: This study is the first to demonstrate that consuming nasturtium specifically increases TAC and decreases ox-LDL in prediabetic patients. The results underscore the antioxidant potential of nasturtium as a functional food or nutraceutical to reduce oxidative stress-driven progression toward type 2 diabetes.

Conclusions: Nasturtium intake enhances antioxidant capacity and may help reduce oxidative stress associated with the development of type 2 diabetes. Further research is needed to confirm its role in T2D prevention. **Trial registration:** NCT05346978, dated 23 April 2022.

Keywords: Pre-diabetes, *Tropaeolum majus L.*, Glucotropaeolin, BITC, Total antioxidant capacity, ox-LDL



Graphical Abstract: Nasturtium (*Tropaeolum majus L.*) sub-chronic consumption modulates oxidative stress biomarker response in prediabetic subjects.

INTRODUCTION

The imbalance between reactive oxygen species (ROS) and the endogenous cellular systems' ability, along with exogenous antioxidants (AOX), to neutralize these reactive molecules is defined as oxidative stress (OS) [1]. OS is implicated in chronic diseases like type 2 diabetes (T2D), where persistent hyperglycemia raises ROS levels, triggering pathways that cause insulin resistance and β-cell dysfunction [2]. Since T2D is often initially asymptomatic, vascular complications and redox imbalance have been detected even during prediabetes [3].

Under normal conditions, OS might be effectively neutralized by enhancing cellular defenses through endogenous production of antioxidant enzymes [4]. In addition, exogenous antioxidants from dietary sources, such as fruits, leaves, and certain edible flowers, further support the antioxidant defense system by neutralizing ROS.

Nasturtium (*Tropaeolum majus L.)* is an edible flower included in the order Brassicales, family *Tropaeolaceae,* native to South America Andean. Nasturtium contains abundant amounts of phenolic compounds such as quinic acid, including caffeoylquinic acid and coumaroylquinic acid derivatives [5-6], and benzyl GSL or glucotropaeolin. Through the hydrolysis facilitated by the enzyme myrosinase, found in either the plant or human gut microbiota [7], glucotropaeolin is converted into the secondary metabolite benzyl isothiocyanate (BITC), which has been associated with anticarcinogenic, anti-inflammatory, and antioxidant properties [8].

Chuang et al [9] showed BITC induced antioxidant protection by the increase in the expression of antioxidant enzymes glutamyl-cysteine ligase modulator (*GCLM*), heme oxygenase 1 (*HO-1*), glutathione S -pi transferase (*GSTP*), and glutathione (*GSH*), in mouse myotubes C2C12 treated with palmitic acid (PA) and BITC. In addition to their antioxidant effects, isothiocyanates (ITCs) reduce NF-κB transactivation, a mediator of the inflammatory response [10]. Waterman et al. [11] showed that supplementation with glucotropaeolin-enriched moringa in mice fed a high-fat diet (HFD) for 3 months reduced interleukin-1β [11] and tumor necrosis factor alpha (*TNF-α*). The effects of isothiocyanates have also been reported in humans [12]. A significant increase in total antioxidant capacity (TAC), a reduction in oxidized low-density lipoprotein (ox-LDL), and oxidative stress index (OSI) were observed in T2D subjects after a sulforaphane (SFN) rich broccoli sprout powder supplementation [12].

Evaluating the effects of bioactive compound intake on human antioxidant status is complicated by the absence of a standardized biomarker for oxidative stress. Intervention studies should use complementary biomarkers reflecting changes in antioxidant capacity and oxidative damage to comprehensively assess redox status [13]. Among these, oxidized LDL (ox-LDL), recognized by EFSA, is useful for measuring lipid oxidative damage [14]. Antioxidant response is often evaluated by measuring antioxidant enzyme expression or activity, and total antioxidant capacity (TAC) is measured using ORAC and ABTS assays [15].

Currently, no studies in healthy humans or those with pathological conditions have addressed the antioxidant effects of nasturtium consumption. Therefore, this paper aims to show the effects of sub-chronic consumption of freeze-dried nasturtium leaves on inflammatory and antioxidant biomarkers in pre-diabetic subjects. This study contributes to understanding the mechanisms by which nasturtium exerts its anti-inflammatory and antioxidant effects, as reported in previous publications.

METHODS

Study Design: This research is a continuation of a previous trial registered at ClinicalTrials.gov (NCT05346978). The first phase of the study assessed, in prediabetic patients, the impact of nasturtium supplementation on insulin resistance and lipid profile biomarkers. The study design and initial findings were outlined in Barrantes-Martínez et al. [16]. The present study assesses the analysis of secondary parameters. This is a clinical, experimental, controlled, double-blind, randomized, crossover pilot study conducted at the Pontificia Universidad Javeriana and the Hospital Universitario San Ignacio (HUSI) in Bogotá, Colombia. All participants provided written informed consent, and all procedures had previously received approval from the Ethics Committee of the Faculty of Science and Medicine. (FM-CIE-0162-19).

Subjects: Ten subjects aged 25 to 70 years were selected to undergo a 2-hour glucose tolerance test, with results ranging from 140 to 199 mg/dl, classified as pre-diabetes. The modest sample size was acceptable for the present study because the randomized crossover design allowed each participant to serve as their own control, thereby minimizing inter-individual variability and enhancing statistical power. A sample of 10 participants is consistent with preliminary, mechanistic, and pilot-level clinical trials in which the primary objective is to detect within-subject changes rather than population-level effects. [17]. The exclusion criteria for the study and additional recruitment information are available in the first phase of this study [16].

Intervention and Monitoring: Figure 1 summarizes the intervention process. One week before the first intervention and during the experimental period, eligible patients were instructed to refrain from eating meals containing GSL-rich vegetables. These vegetables included members of the Brassica family, such as broccoli, Brussels sprouts, cabbage, cauliflower, and radish, as well as *Moringa oleifera*. Participants were instructed to continue their usual dietary and lifestyle practices throughout the intervention period, except for consuming *Brassica* vegetables and *Moringa.*

Participants were randomly assigned to two groups: the placebo group (PLC, n=10) and the nasturtium group (NT, n=10). Following a 4-week intervention period, the treatment assignments were reversed: the NT group received the placebo, and the PLC group received nasturtium. Details of the intervention procedures with NT and PLC are described in the first phase of the study [16]

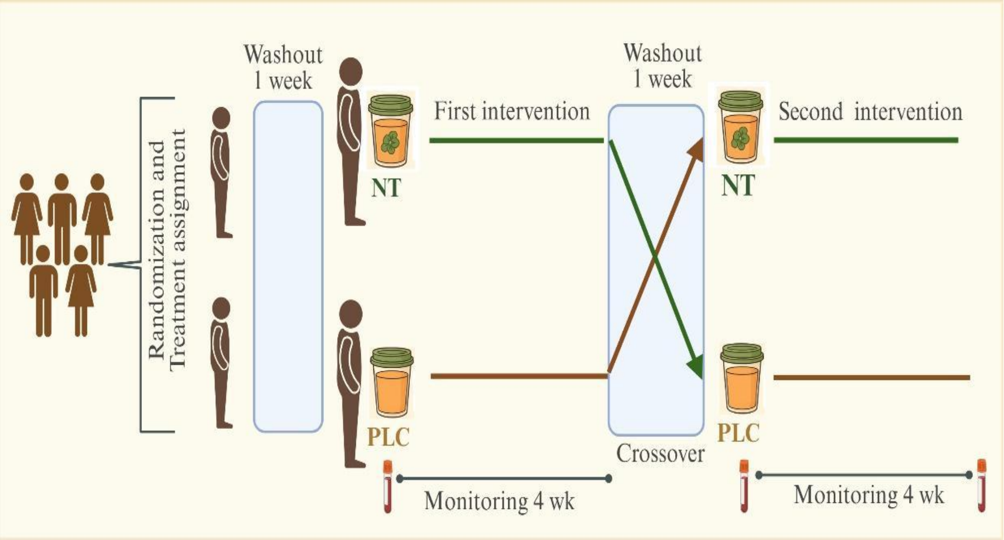


Fig. 1. Study design, treatment, and sampling timeline. PLC, Placebo; NT, Nasturtium. Created in https://BioRender.com

Participants attended three visits to the HUSI clinical laboratory for primary and secondary outcome measurements: at baseline (week 0) and after the NT and PLC interventions (week 4). Weekly follow-ups monitored compliance, gastrointestinal symptoms, physical activity, and consumption of glucosinolate (GSL)-rich foods. A GSL-focused food frequency questionnaire assessed food consumption, while physical activity levels were measured using the short international physical activity questionnaire (IPAQ). The details of the anthropometric evaluation were described in the first phase of the study [16]. Participants sent photographic evidence of empty envelopes and bottles to confirm 100% compliance, with no withdrawals.

Nasturtium and Placebo Drinking Description: The source of the vegetable material for supplementation was described in Barrantes-Martínez et al. [16]. The GSL content was determined using the conditions defined by Koo et al. [18]. Per gram, there were 54 µmol of benzyl GSL and 44 µmol of BITC. The 15 grams/week of NT administered to each participant provided 810 µmol of benzyl GSL or 681 µmol of BITC, respectively. The placebo consisted of 3 grams of hydrolyzed collagen (Solugel® collagen peptide). Both sachets were packaged identically and protected from light. The proximal composition of NT and PLC was described in Barrantes-Martínez et al [16].

Sample Collection and Biomarker Analysis: After a 12-hour fasting period, blood samples were obtained from the antecubital vein. Serum and plasma were then collected in Vacutainer® tubes (BD Vacutainer®) for the analysis of specific biomarkers. All samples were gathered at the HUSI Clinical Laboratory.

Gene Expression: Peripheral blood mononuclear cells (PBMC) were isolated from defibrinated blood collected in EDTA vacutainer tubes by employing the Ficoll-Hypaque density gradient method (Sigma, USA). The RNeasy Mini Kit (Qiagen, Hilden, Germany) was used for RNA isolation, and RNA concentrations were determined using a NanoDrop 2000C spectrophotometer (Thermo Scientific, USA) with a 260/280 OD ratio between 1.8 and 2.0 to assess DNA integrity. The OneScript®Plus cDNA synthesis kit (Thermo Fisher Scientific, USA) was used for RT-PCR. The sensi FAST SYBR No-ROX KIT (Meridian Bioscience, USA) and BIO-RAD CFX96 equipment with CFX master software were used for real-time PCR amplification, and the analysis was repeated at least three times. Gene expression was calculated using a comparative method ($2^{-\Delta\Delta Ct}$) [19]. The genes evaluated and the primers used for gene expression are listed in Table 1.

Table 1. Genes and primer sequence for gene expression analysis

Gene	Forward 5 -> 3	Reverse 5 -> 3
IL-1β	CCACAGACCTTCCAGGAGAATG	GTGCAGTTCAGTGATCGTACAGG
IL-6	AGACAGCCACTCACCTCTTCAG	TTCTGCCAGTGCCTCTTTGCTG
IL-2	AGAACTCAAACCTCTGGAGGAAG	GCTGTCTCATCAGCATATTCACAC
IL-10	TCTCCGAGATGCCTTCAGCAGA	TCAGACAAGGCTTGGCAACCCA
CAT	GTGCGGAGATTCAACACTGCCA	CGGCAATGTTCTCACACAGACG
SOD2	CTGGACAAACCTCAGCCCTAAC	AACCTGAGCCTTGGACACCAAC
NQO1	CCTGCCATTCTGAAAGGCTGGT	GTGGTGATGGAAAGCACTGCCT
GCLC	GGAAGTGGATGTGGACACCAGA	GCTTGTAGTCAGGATGGTTTGCG
GCLM	TCTTGCCTCCTGCTGTGTGATG	TTGGAAACTTGCTTCAGAAAGCAG
GAPDH	CATCAATGGAAATCCCATCA	TTCTCCATGGTGGTGAAGAC

IL-1β, interleukin-1β; *IL-6*, interleukin 6; *IL-2*, interleukin-2; *IL-10*, interleukin 10; *CAT*, catalase; *SOD2*, superoxide dismutase-2; *NQO1,* NAD(P)H:quinone oxidoreductase-1; *GCLC*, glutamate-cysteine ligase catalytic subunit; *GCLM*, glutamate-cysteine ligase modifier subunit; housekeeping gene *GAPDH,* Glyceraldehyde 3-phosphate dehydrogenase.

Oxidized Low-Density Lipoprotein Detection: Serum ox-LDL levels were measured using an ELISA kit (Novus Biologicals, USA) according to the methodology described by Bülbül Ayci et al. [20] with an intra-assay coefficient of variation <10% (mean, 6.43%) for most samples. One sample showed a coefficient of variation >10%.

Total Antioxidant Capacity (TAC): For ORAC and ABTS methodologies the following reagents were used: methanol (MeOH), acetone (Ace), acetonitrile (ACN), sodium fluorescein (FL), phosphate buffer (PBS) prepared from phosphate potassium acid (K_2HPO_4) and potassium phosphate monobasic (KH_2PO_4), 2,2'-azobis dihydrochloride(2-amidinopropane) (AAPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and Potassium persulfate ($K_2S_2O_8$) were obtained from Sigma-Aldrich (Madrid, Spain). 2,2′-azino-bis (3-ethylbenzothiazoline-6-diammonium sulfonate (ABTS) was obtained from BIOBASIC (Markham, ON, Canada). Ultrapure water was obtained from a Millipore system (Billerica, MA, USA).

Blood samples were centrifuged at 1500 g for 10 min. A sterile Pasteur pipette was used to aspirate the supernatant, which was stored in Eppendorf tubes at -80°C until processing. The samples were deproteinized according to the procedure outlined by Crowe-White et al. [21].

TAC determination by ORAC: The ORAC determination was based on the method proposed by Benítez-Estrada [22]. Initially, PBS 0.075 M was used to dilute all the reagents. The protocol involved the use of (i) 165 µl of the fluorescein stock solution (0.44 mg/mL and pH 7.0) in 25 mL of PBS, (ii) a solution of AAPH at 60 mg/mL, and (iii) a solution of Trolox standard at 100 µM. The solutions were kept in the dark at -20°C until utilization. A plate reader set to an excitation wavelength of 485 nm and an emission wavelength of 535 nm was used to measure total antioxidant capacity (TAC). Readings were taken at 5-minute intervals for 3.5 hours, with linear shaking performed before each measurement. The ORAC value was determined by calculating the difference in the area under the curve (AUC) representing the reduction in fluorescence over time [22], as detailed below: The area under the curve (AUC) was measured:

$$\text{AUC: } 0.5 + (f1/f0 + f2/f0 + f3/f0 + f4/f0 + \ldots + f34/f0 + f35/f0)$$

Where:

- f0 is the fluorescence reading taken at 0 minutes.
- fi is the fluorescence reading taken at time i.

The AUC of the samples was subtracted from the AUC of the blank. The results were expressed as Trolox ET in µmol per liter of sample [22].

TAC determination by ABTS: For ABTS determination, the methodology of Limsuwan et al [23] was used with some modifications, as is shortly described: i) 0.075 M phosphate buffer at pH 7.0, ii) Trolox solution at 2.5 mM in PBS, iii) 7 mM aqueous ABTS solution in PBS, and iv) 140 mM potassium persulfate solution in PBS. 40 mL of an aqueous ABTS solution was mixed with 704 µl of potassium persulfate (2.45 mM). The mixture was incubated for 16 hours in the dark at room temperature, then diluted with PBS to achieve an absorbance of 744 nm at 0.7 ± 0.05, with additional shaking. A total of 1450 µL of the ABTS cation radical aqueous solution, 25 µL of the standard or sample post-deproteinization, and 25 µL of the PBS solution were combined in an Eppendorf tube and stirred for 30 s. A 250 µL aliquot was extracted and placed on a plate. Absorbance values were recorded at a wavelength of 744 nm at both the initial time (0 min) and 60 min after radical neutralization. PBS served as the blank. The results are expressed as µmol Trolox/100 mL sample.

Secondary Outcome: A correlation analysis was performed between the biochemical biomarkers evaluated during the first phase of the study [16] and the gene expression of factors described in Table 1.

Statistical Analysis: Data analysis was conducted using SPSS statistical software (version 21; SPSS, Inc., Chicago, IL, USA). To assess the normality assumptions regarding gene expression and total antioxidant capacity, the Shapiro-Wilk test was used. Group differences in gene expression were evaluated using ANOVA or the Friedman test. The percentage difference in TAC was calculated as [(4-week values – initial values)/initial values × 100] and analyzed using the T-test or the Wilcoxon test. For the correlation analyses, Pearson or Spearman correlation coefficients were employed. A significance level of $p < 0.05$ was deemed to indicate statistical significance.

Results: All 10 participants completed the clinical trial. The average age was 51 ± 11 years, with 80% of participants being women. No changes in anthropometric characteristics were observed following either the placebo or nasturtium intervention. Analysis of renal, hepatic, and hematological function showed no adverse effects or noteworthy variations between the groups. However, some participants experienced gastrointestinal manifestations after treatment with nasturtium, including nausea (60%), bloating (30%), and a discomforting spicy taste (20%). The food frequency questionnaire showed that none of the participants consumed GLS-rich foods during the follow-up.

Gene expression analysis: The ANOVA analysis revealed no significant differences in gene expression profiles related to anti- or pro-inflammatory responses between intervention groups. A minor level of significance was observed for *CAT* (P = 0.045), with non-significant trends for *NQO1, SOD, GCLC*, and *GCLM*. Correlation analysis between gene expression, lipid profile, and cardiovascular risk indices showed significant differences across groups (Table 2)

Table 2. Correlation analysis between gene expression patterns and biochemical biomarkers within groups.

Nasturtium			**Placebo**			**Baseline**		
Gen- Biomarker	**R**	***p. value***	**Gen- Biomarker**	**R**	***p. value***	**Gen- Biomarker**	**R**	***p. value***
IL-1β - ALT	0.8	0.01*	*IL-2* - oxLDL	0.714	0.026*	*IL-2* – oxLDL	-0.885	0.003**
IL-1β -Insulin	0.933	0***	*CAT*- oxLDL	-0.844	0.008**	*IL-10* - AST	-0.657	0.039*
IL-10 - TC	-0.697	0.025*	*SOD* - LDL	-0.721	0.019*	*IL-6*- LDL	-0.721	0.019*
IL-10 - LDL	-0.709	0.013*	*SOD* - LDL/HDL	-0.818	0.004**	*CAT* - oxLDL	-0.779	0.023*
IL-10 - oxLDL	-0.762	0.028*	*IL-10* - Insulin	0.685	0.029*	*SOD*-LDL	-0.806	0.005**
IL-10 - TC/HDL	-0.782	0.008**	*IL-10* - HOMA	0.709	0.022**	*SOD*-AST	-0.796	0.006**
IL-10 -LDL_HDL	-0.806	0.005**	*IL-6* - Insulin	0.721	0.019*	*SOD* - oxLDL /LDL	0.738	0.037*
IL-10 -AC	-0.782	0.008**	*IL-6* - HOMA	0.709	0.008**	*NQ0 1*- LDL	-0.782	0.008**
IL-6 – TC/HDL	0.879	0.001**	*IL-6* – LDL/HDL	-0.673	0.033*	*GCLM* - Insulin	0.723	0.018*
IL-6 - *LDL/*HDL	-0.915	0***	*GCLC* - AST	0.695	0.038*	*GCLM* - HOMA	0.697	0.025*
CAT- oxLDL	-0.802	0.017*	*GCLM* - TG	0.733	0.025*	*GCLM* - GGT	0.822	0.005**
SOD - TC	-0.673	0.033*	*GCLM* - AC	0.717	0.03*			
SOD - LDL	-0.818	0.004**	*GCLM* -TC/HDL	0.717	0.03*			
GCLC- LDL	-0.661	0.038*						
GCLC-LDL/HDL	-0.745	0.013*						
GCLM – TC	0.952	0***						
GCLM - LDL	0.891	0.001**						
GCLM - oxLDL	0.833	0.01*						

Correlation analyses between gene expression patterns and biochemical biomarkers within groups. Pearson or Spearman correlation coefficients were applied as appropriate. Significance levels reflect the magnitude of the correlation significance within the group and are indicated as follows: *P < 0.05, **P < 0.01, ***P < 0.001. ALT, alanine aminotransferase; HOMA, Homeostatic Model Assessment for Insulin Resistance; Castelli’s risk index I and II (TC/HDLc and LDLc/HDLc); TC, total cholesterol; LDL, low density lipoprotein; ox-LDL, oxidized-low density lipoprotein; AC, Atherogenic coefficient (non-HDLc/HDLc); AST, Aspartate aminotransferase; GGT,γ-glutamyl transferase; GCLC, glutamate-cysteine ligase catalytic subunit; GCLM, glutamate-cysteine ligase.

Antioxidant and oxidative biomarkers analysis

Figure 2 shows the mean changes in oxidative stress biomarkers from baseline for both nasturtium and placebo treatments. Nasturtium significantly increased TAC by 21% (ORAC) and 10% (ABTS), and reduced ox-LDL by 13% (P < 0.05) compared to PLC. A significant negative correlation was observed between ox-LDL and ORAC values (r = -0.848, P < 0.001), suggesting that ox-LDL reduction is associated with increased antioxidant capacity.

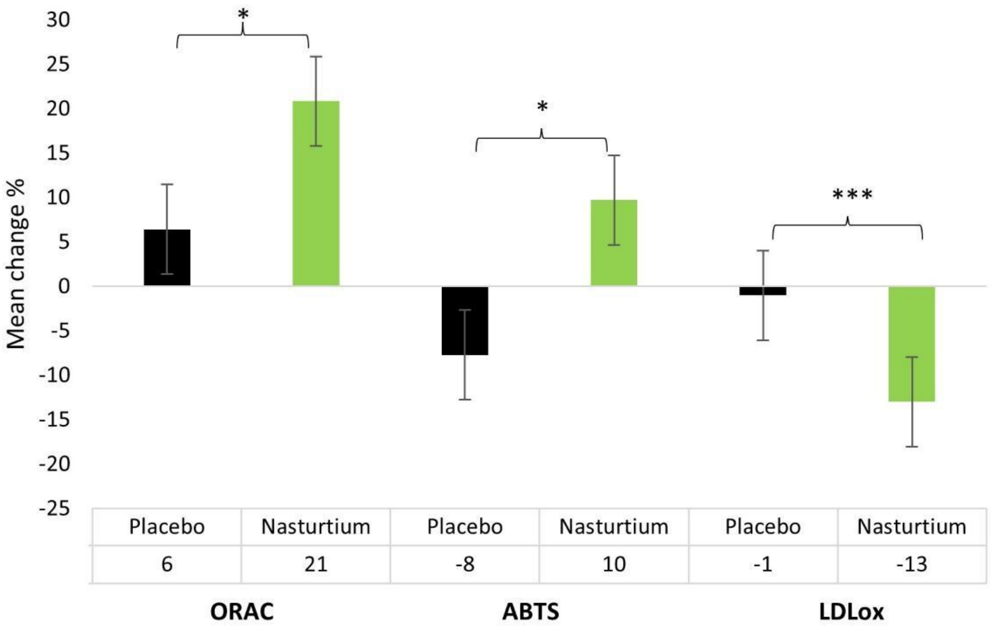


Figure 2. Mean percentage change in the groups relative to baseline values across both treatments. All values are mean percentage ± SEM, *Significantly different compared with baseline values according to the formula [(4-week values - baseline values)/baseline values × 100] ($p < 0.05$). *** significant difference compared with baseline values ($P < 0.001$).

DISCUSSION

This study evaluated the impact of a 4-week course of weekly consumption of a nasturtium drink on gene expression of cytokines and antioxidant enzymes, TAC activity (measured by ORAC and ABTS), and oxidative stress (ox-LDL) in adult prediabetic subjects. Nasturtium bioactive compounds are known for their chemopreventive effects [24] and for modulating lipid and carbohydrate metabolism, antioxidant capacity, and the anti-inflammatory response [25]. These effects support their potential role in preventing nutrition-related chronic diseases such as T2D.

According to our results, comparing gene expression between participants treated with nasturtium and those treated with the placebo did not reveal a significant difference. The cytokine and antioxidant enzyme markers were selected based on prior studies demonstrating in vivo effects of isothiocyanates or phenolic compounds from edible plants [26]. The lack of effect may be due to the small sample size, which limited control over individual variability, as well as the timing, frequency, and dose of the intervention. While no previous studies have examined the effects of sub-chronic nasturtium consumption on these gene markers in humans, similar effects have been reported with other glucosinolate-rich plants.

Hofmann et al. [27] administered 85g of gluconasturtin-rich watercress daily for eight weeks to healthy subjects and found no significant changes in PBMC gene expression of antioxidant enzymes SOD2 and GPX1. In contrast, Fahey et al. [28] reported increased PBMC gene expression related to cytoprotection and antioxidant functions after a single dose of glucoraphanin-rich broccoli seed and sprout extract. Probably longer exposure (≥4 weeks) to these compounds may better reveal effects on inflammatory and antioxidant markers. Participants' health conditions, such as prediabetes, likely influenced the expression of several genes. As has been shown previously, glucolipotoxicity in T2D promotes inflammation, OS, insulin resistance, and beta-cell failure [29]. In the present study, participants showed variability in glucose, lipid, and insulin profiles, as well as in body fat mass [16]. Dai L et al. [30] reported disease consequences on PBMC gene expression variability. Although prediabetes offers an intervention point without glucose-lowering drugs, associated excess body fat, inflammation, and lipid disturbances may have affected gene expression. Despite no significant group differences, correlation analyses between gene expression and metabolic biomarkers reflected metabolic adaptation mechanisms in prediabetes. Positive correlations were found between *IL-6* and TC/HDLc, and *IL-1β* with ALT, insulin, and HOMA-IR, consistent with other studies [31]. As widely reported, chronic hyperglycemia and obesity trigger low-grade inflammation, increasing proinflammatory markers IL-6, IL-1β, and TNFα, as well as ROS production [32].

The inverse correlation between IL-10 and SOD gene expression and the lipid profile, ox-LDL, and cardiovascular risk indices reflects the counter-regulatory response of antioxidant enzymes and anti-inflammatory cytokines during chronic low-grade inflammation, often enhanced by bioactive dietary compounds. Chuang et al. [9] showed that BITC upregulated antioxidant enzymes, including HO-1, GSTP, and GSH, thereby reducing oxidative stress in muscle cells and mice. Correlation results for *GCLC* and *GCLM* genes were mixed: *GCLM* positively correlated with lipid profile and risk indices, whereas GCLC negatively correlated with LDL and the Castelli II index. Since GCLC and GCLM subunits produce GSH, and BITC is mainly metabolized bound to GSH, intake of GSL or BITC derivatives is expected to increase their expression [33]. Although in vitro studies show positive regulation by GSLs and phenolics [33], the effect of nasturtium in humans has not yet been established. It may be influenced by genetic polymorphisms that affect *GCL* subunit expression or activity [34]. Oxidative stress can activate these subunits independently of *GCL* expression, but mechanisms remain unclear [35]. More research is needed on their role in the dietary prevention of T2D. After nasturtium intervention, ox-LDL decreased by 13%, while TAC increased by 21% (ORAC) and 10% (ABTS), with a strong negative correlation between ox-LDL and ORAC-measured TAC. These benefits may result from: (i) BITC activating Nrf2 to boost antioxidant/detox enzymes and reduce proinflammatory NF-κB [10]; (ii) phenolics enhancing GSH, SOD, GST, activating Nrf2 [36], and modulating NF-κB and iNOS in the reduction of inflammatory response [37-38]; and (iii) vitamin C's antioxidant effects [39]. Although sub-chronic nasturtium effects on TAC lack direct evidence, similar increases in TAC have been observed with other GSL-rich vegetables [40]. Bahadoran et al. [12] observed reduced MDA, ox-LDL, and the oxidative stress index, alongside increased TAC, following consumption of broccoli sprout powder. In prediabetes and diabetes, elevated oxidative stress promotes LDL oxidation, activates NF-κB, and contributes to atherogenesis. The significant reduction in ox-LDL here suggests that nasturtium may protect against ox-LDL-induced LDL lipid peroxidation and inflammation [41].

This study is the first to demonstrate that nasturtium consumption increases total antioxidant capacity (TAC) and decreases oxidized LDL (ox-LDL) in prediabetic patients, highlighting its antioxidant potential to mitigate the progression to T2D driven by oxidative stress. However, it has some limitations. The limited sample size typical of crossover designs may have limited the ability to control individual variability, potentially resulting in non-significant findings in gene expression. Additionally, preventing participants from using medications for glycemia, lipids, or hypertension hindered recruitment efforts. Furthermore, limited blood sampling points may not fully reflect baseline conditions after the washout, though the 7-day washout was deemed appropriate based on bioactive compound clearance. [42]. Most participants were women aged 40 or older, reflecting the demographic distribution of the accessible study population. The TD2 prevalence in Latin America and in the world is slightly higher in women, and it is diagnosed regularly after the age of 30 years old [43]. The majority presence of women in the study is justified because the intervention was not expected to produce sex-specific physiological responses relevant to the primary endpoints. However, the power to analyze sex-by-treatment interactions, and any observed effects, for instance, gastrointestinal manifestations [44], may not be generalizable to a more balanced or male-dominant population. As a result, while the findings provide valuable preliminary insights, they should be interpreted with caution, and future studies with larger, more sex-diverse samples will be necessary to determine whether the intervention elicits differential effects across sexes.

Scientific Innovation and Practical Implications: This study represents the first clinical trial to examine the effects of subchronic consumption of a freeze-dried nasturtium drink on antioxidant and inflammatory biomarkers, specifically in prediabetic subjects. Given that phytochemicals in nasturtium are recognized for their antioxidant and anti-inflammatory effects, this study demonstrates that regular consumption of nasturtium leaves significantly enhances total antioxidant capacity in a prediabetic population. Notably, the study highlights the potential of nasturtium as a functional food, dietary supplement, or nutraceutical that could help attenuate OS associated with chronic conditions such as T2D.

CONCLUSION

This study demonstrated that intake of 15 g of nasturtium per week for 4 weeks significantly increased TAC, as measured by the ORAC and ABTS methodologies, and reduced ox-LDL in prediabetic patients. Since gene expression may be influenced by a myriad of metabolic factors secondary to an individual's health status, further studies with larger sample sizes are warranted to elucidate the effects of nasturtium supplementation on gene and protein biomarkers associated with immune and antioxidant responses.

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Data Availability Statement: The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Approval: This study was conducted in accordance with the guidelines of the Declaration of Helsinki, and all procedures involving research participants were approved by the Ethics Committee of the Faculties of Sciences and Medicine, Javeriana University (FM-CIE-0162), dated April 22, 2016. Written informed consent was obtained from all participants after explaining the study purpose and ensuring privacy and confidentiality.

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