Journal of Ethnopharmacology 318 (2024) 116862

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

The hydroalcoholic extract of *Nasturtium officinale* reduces oxidative stress markers and increases total antioxidant capacity in patients with asthma

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ARTICLE INFO

Keywords:
Asthma
Nasturtium officinale
Clinical trials
Cytokines
Malondialdehyde
Superoxide dismutase
Tumor Necrosis Factor α

ABSTRACT

Ethnopharmacological relevance: Asthma is a common chronic disease characterized by inflammation of the airways. One of the most devastating consequences of this inflammatory process is the production of reactive oxygen species responsible for oxidative stress.

Nasturtium officinale commonly known as watercress has traditionally been applied in Iranian folk medicine to treat respiratory disorders and diseases mainly bronchitis and asthma. In accordance with these ethnopharmacological reports, through our previous in vivo experiment, we have confirmed significant effect of its hydroalcoholic extract in reducing lung inflammation and oxidative stress in an ovalbumin-induced asthmatic rat model.

Aim of the study: The aim of the present study was to investigate the anti-inflammatory and antioxidant effects of *N. officinale* hydroalcoholic extract (NOE) in patients with asthma, in order to confirm our findings of the previous performed in vivo study.

Material and methods: The NOE capsules (500 mg) were treated twice daily for 4 weeks as a supplementary treatment in a randomized, double-blind, and placebo-controlled trial in asthmatics. The primary outcome was Asthma Control Test score. The blood samples were taken at the beginning and end of the study. Then, the level of inflammatory markers, oxidative stress markers and antioxidant enzyme activity were measured.

Results: Treatment with NOE for one month caused a reduction in the levels of MDA, PCO and NO metabolite markers compared to the placebo group. In addition, FRAP levels as an indicator of total antioxidant capacity in the intervention group was significantly increased at the end of the treatment period compared to pre-treatment values.

Conclusion: Findings demonstrated that NOE may have a therapeutic effect on asthma by improving oxidative stress. However, more studies are required to support these results. Moreover, bio-assay guided fractionation and isolation approach can be conducted to identify major bioactive compound/s.

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https://doi.org/10.1016/j.jep.2023.116862
Received 12 August 2022; Received in revised form 28 February 2023; Accepted 27 June 2023
Available online 10 July 2023

1. Introduction

Asthma is one of the most common chronic disease worldwide (Masoli et al., 2004). Currently over 315 million people in the world and about 12–14% of the Iranian's population suffer asthma (Khaldi et al., 2018; Varmaghani et al., 2016). Asthma is characterized by reversible obstruction and stenosis, severe response, severe inflammation, and altered airway structure. These changes include epithelial surface metaplasia, goblet cell hyperplasia and increased mucus secretion, subepithelial fibrosis, increased smooth muscle mass due to cell hyperplasia, as well as hypertrophy and increased number and diameter of vessels (Huang et al., 2017; Martin and Tamaoka, 2006). Inflammation of the airways is associated with the reaction of various immune cells and different mediators, which ultimately leads to pathophysiological changes in asthma. Bronchitis and airway obstruction lead to clinical manifestations of cough, wheezing, and shortness of breath (Antunes et al., 2020; Salehi et al., 2011). Also, as a result of the inflammatory process, with the migration of inflammatory cells, specially eosinophils and mast cells under the mucosa and epithelium, their number increases in the submucosal layer and lavage fluid of the respiratory tract (Huang et al., 2017; Martin and Tamaoka, 2006). These changes are mediated by inflammatory mediators, including cytokines such as chemokines, interleukins, and lipid mediators such as leukotrienes and prostaglandins, which are produced by inflammatory and non-inflammatory cells (Halwani et al., 2010).

Abbreviations

NOE	*Nasturtium officinale* extract
ACT	Asthma Control Test
SD	Standard deviation
TNF-α	Tumor Necrosis Factor α
PCO	Protein carbonyl
T-SH	Total thiol
MDA	Malondialdehyde
CAT	Catalase
ROS	Reactive oxygen species
NF-KB	Nuclear factor-kappa B
IL-1β	Interleukin-1β
ICAM-1	Intercellular adhesion molecule-1
VCAM-1	Vascular adhesion molecule-1
COX2	Cyclooxygenase-2
NO	*Nasturtium officinale*

Reactive oxygen species (ROS) are produced in the response to many physiological conditions during the normal functioning of the human body. However, ROS production causes severe cell damage (Sagols and Priymenko, 2011). Due to the inflammatory nature of asthma, the role of oxidative stress has been proposed systematically and locally in the pathogenesis of this disease (Nadeem et al., 2003). Infiltration of inflammatory cells such as macrophages and eosinophils in the airways of patients can produce various mediators that are responsible for causing inflammatory reactions in asthma. The complex reaction between cells and mediators leads to the increased ROS production (MacPherson et al., 2001). The ROS impact on the asthmatic lungs include increased lipid peroxidation and airway sensitivity, as well as secretion and increased vascular permeability (Rajizadeh et al., 2019; Zhang et al., 2018). In addition, during persistent asthma and asthma attacks, the antioxidant capacity of serum decreases dramatically, therefore a lack of antioxidant in the airway of asthmatic patients can exacerbate ROS imbalances, consequently, enhance the inflammatory response of the patients (Zhang et al., 2018).

Airway abnormalities, including the increased production of various cytokines, chemokines, and grow factors initiate activation of intracellular signaling pathways. There are many pathways associated with signaling molecules and their role in the asthma development. One of these pathways is the nuclear factor-kappa B (NF-κB) (Mishra et al., 2018; Yuan et al., 2019). NF-κB is an important regulator in many cellular processes, including the control of inflammatory responses, immune processes, proliferation, cell differentiation, and apoptosis (Yan and Choi, 2014). This factor is activated by many parameters including inflammatory cytokines such as interleukin-1β (IL-1β), Tumor Necrosis Factor α (TNF-α), and ROS, subsequently causes the production of inflammatory proteins comprising intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1(VCAM-1), and cyclooxygenase-2 (COX2) (Duan et al., 2018). These results suggest that inhibition of TNF-α activity to reverse inflammation and regenerate airway may be a potential strategy for asthma management.

Many patients are prescribed short-and long-term corticosteroids and B-adrenergic agonists or leukotrienes for the treatment; however, 5–10% of the patients fail with this treatment.

The use of herbal medicines as a supplement to standard therapies is useful in many chronic conditions such as asthma (Inam et al., 2017). *Nasturtium officinale* R.Br. is the scientific name (http://www.theplantlist.org/tpl1.1/record/kew-2381026) for watercress, a species of aquatic and perennial plant belonging to the Brassicaceae family. *N. officinale* contains a number of vitamins such as A, B, C, E, K and folic acid and some of the body's active ingredients including β-carotene, lutein, and quercetin. The *N. officinale* ethanolic extract (NOE) demonstrated strong antioxidant activity, while this activity has been attributed to various factors such as inhibition of the free radical chain initiation, binding to metal ion catalysts, decomposition of peroxides and inhibition of hydrogen decomposition (Jeon et al., 2017; Ozen, 2009). Previous studies showed that *N. officinale* is applied for the treatment of diabetes, bronchitis, influenza, diuresis, and asthma (Azarmehr et al., 2019; Sadeghi et al., 2019). In another study, about 20 compounds were identified from the *N. officinale* roots, which were coumaric acid and its derivatives, synaptic acid, caffeic acid, beside phenolic compounds. The amount of phenolic compounds in the plant methanolic extract was higher than its aqueous extract (Zeb, 2015). In other studies, phytochemical analysis of the *N. officinale* extracts showed that glucosinolates (Engelen-Eigles et al., 2006), flavonoids and phenolics are the main compounds (Gill et al., 2007). In addition, anti-diabetic (Mousa-Al-Reza Hadjzadeh et al., 2015), anti-cancer (Schuchardt et al., 2019), anti-inflammatory and antioxidant (Boligon et al., 2013; Spínola et al., 2017) effects of *N. officinale* have been reported in various preclinical and clinical studies. In Iranian traditional medicine, *N. officinale* is used to treat bronchitis, tuberculosis, influenza, and asthma (Sedaghattalab et al., 2021), furthermore, we confirmed in our previous study that the hydroalcoholic extract of *N. officinale* reduced lung inflammation and oxidative stress in an ovalbumin-induced rat model of asthma (shakerinasab et al., 2022). Considering the role of oxidants in asthma and airway inflammation and the antioxidant and anti-inflammatory effects of NOE, the aim of the present study was to investigate the anti-inflammatory and antioxidant effects of the *N. officinale* hydroalcoholic extract in asthmatic patients.

2. Materials and methods

2.1. Design

The study was designed as a randomized, double-blinded, placebo-controlled clinical trial. This clinical trial was registered in the Iranian Registry of Clinical Trial (IRCT Code: IRCT2014101519546N1) and was also approved by the Ethical Committee of the Yasuj University of Medical Sciences (Code: 910683) and conducted in accordance with the Declaration of Helsinki (Association, 2009). All patients provided written informed consent before participating in the study.

2.2. Participants

The eligibility of 60 moderate asthmatic patients were assessed based on Global Initiative for Asthma (GINA) guideline (Global Initiative for Asthma, 2014) (Koshak et al., 2017) in the Shahid Mofattah Clinic, Yasuj. All patients received routine treatments including salbutamol, salmeterol or formoterol, budesonide or fluticasone, and montelukast. The inclusion criteria for patients were: adult male/female (age 18–65 years), asthma diagnosis based on the GINA, asthma symptoms not fully controlled based on ACT (Asthma Control Test) score from 5 to 24 (Koshak et al., 2017; Sigari et al., 2011), no severe asthma exacerbation in the last four weeks, and able to obtain consent. The exclusion criteria were serious co-morbid conditions, smoking history, pregnant women, taking any preparation containing NOE, and known history of hypersensitivity to NOE.

2.3. Preparation of drug and placebo

Aerial part of *N. officinale*, including stems and leaves were collected from Kakan region located in Yasuj during the summer of 2020. The plant was identified by the botanist (Dr. Azizollah Jafari), where a voucher specimen (herbarium number HYU30230) was deposited at the Yasuj University, faculty of science. After collecting the plant samples, they were cleaned and kept away from direct light in the room air for several days to dry, crushed and ready to be extracted. Thus, 100 g of the dried drug was soaked in 500 mL of solvent (70% ethanol). The resulting mixture was placed at 37 °C for 48 h. Subsequently, the yielded hydroalcoholic extract was filtered, and the solvent was totally vaporized under reduced pressure via a rotavapor at 40 °C and the powdered extract of *N. officinale* was consequently obtained. The powdered drug was then encapsulated (500 mg). Flour was selected as a Placebo and encapsulated identically (Azarmehr et al., 2019).

2.4. Study protocol

Patients were randomized to one of two groups: NOE or placebo. Both the investigator and the patients were blinded to the treatment or placebo groups. A randomization list was generated at www.sealedenvelope.com, in blocks of random size (2 or 4). The list was generated by a researcher who did not take part in randomization or patient assessment. The list was uploaded to software for allocation concealment.

The study duration of each patient was four weeks. The dose of NOE capsules recommended for patients was 500 mg twice daily. During the first visit and after signing the study consent form, patients' baseline demographics, symptoms, co-morbid conditions, and medications were recorded. Thereafter, the relevant study outcomes were evaluated, and the ACT was scored by the principal investigator through direct questioning of the patients. Then, the study medication was dispensed to the patients. After four weeks, the patients returned for follow up and were assessed for completion of the outcomes. During the treatment period, the principal investigator contacted patients by phone after the first week to check compliance and any appearance of side effects.

2.5. Blood sampling and biomarkers measurement

Venous blood samples were taken to measure oxidative stress and inflammatory parameters. Furthermore, the ACT test were performed via questionnaire (beginning and end of the study) and pulmonary function test (Koshak et al., 2017).

2.6. Oxidative stress markers

2.6.1. Determination of malondialdehyde (MDA)

The MDA level was determined based on the previous method (Arya et al., 2019). This method was used to assess the color created from the reaction between TBA (thiobarbituric acid) and MDA at 535 nm. The MDA content was quantified using a molar absorption coefficient of 15, 600 M^{-1} cm^{-1} and reported as mmol/L (Alavinezhad et al., 2020).

2.6.2. Determination of nitric oxide metabolite (NO)

The serum level of nitrite metabolite was assessed by Griess reagent method using a standard enzyme-linked immunosorbent assay (ELISA) kit (Arya et al., 2019).

2.6.3. Determination of total thiol (T-SH)

The total thiol content was analyzed by spectrophotometry at 412 nm. Briefly, 25 mL of plasma was mixed into a micro tube with 150 mL of the Tris-EDTA (ethylenediaminetetraacetic acid) buffer followed by adding 10 mL DTNB (10 mM) and 790 mL of absolute methanol. The test tube was put at normal temperature for 15 min and the absorbance was ascertained against DTNB blank and blank tube. The total T-SH was computed using the molar absorption coefficient of 13,600 M^{-1} cm^{-1} (Azarmehr et al., 2019).

2.6.4. Protein sulfhydryl and carbonyl measurement

The plasma protein sulfhydryl were measured at 412 nm by using Ellman's reagent according to the previous study (Sadeghi et al., 2019). The content of protein-bound carbonyls in plasma, an indicator of protein oxidation, was determined at 380 nm by using 2,4-dinitrophenylhdrazine (DNPH) according to the previous study (Doustimotlagh et al., 2014).

2.6.5. Determination of the ferric reducing antioxidant power (FRAP)

The plasma total antioxidant capacity was determined according to previous study (Benzie and Strain, 1996). This method is based on the ferric reducing ability of plasma (FRAP) which is estimated from the reduction of a Fe- Tripyridyl-S-triazine (TPTZ) complex to the ferrous form at low pH. Absorbance of the resulting blue color was measured at 593 nm, and the total antioxidant capacity of plasma was determined using a standard curve.

2.7. Determination of antioxidant enzymes

Measurement of glutathione peroxidase (GPX), superoxide dismutase (SOD), and catalase (CAT) activities were performed with commercial colorimetric assay kits (Zell Bio GmbH, Ulm, Germany) using spectrophotometry (Alavinezhad et al., 2020).

2.8. Assessment of serum levels of cytokines

The plasma samples were employed for IL-1B and TNF-α measurement using a commercial ELISA kit (Zell Bio GmbH, Ulm, Germany) based on the manufacturer's instructions. The Absorbance was read at 450 nm and the standard curves were plotted (Alavinezhad et al., 2020).

2.9. Data analysis

Statistical analysis was performed using SPSS version 21. The normality of quantitative data was evaluated using K–S test. Independent *t*-test was used to compare the mean of normal variables before the intervention between two groups and paired *t*-test was used to compare the score of before and after variables in each group. In case of abnormal data distribution, Wilcoxon test was used, whereas the Mann-Whitney test was exploited to compare two groups. $p < 0.05$ was statistically considered significant.

3. Results

Sixty moderate asthmatic patients aged 18–65 years, participated in this clinical investigation, out of which 48 completed the study. Twenty six patients in the placebo group and 22 patients in the intervention

group (treated with 500 mg/kg/day) were assessed.

Baseline characteristics of the subjects including demographics, oxidative stress, and inflammatory markers are illustrated in Table 1. We found that the percent changes of these variables were not significant between the intervention and control groups.

The comparison of changes in the inflammatory markers during the study period between the control and intervention groups was presented in Table 2. Although, we did not observe any substantial change in the serum levels of inflammatory factors between two groups, the IL-1 was significantly increased in the intervention subjects, and showed a remarkable change compared to the placebo group ($P < 0.05$).

As depicted in Table 3, the significant changes were observed in some of the oxidative and antioxidant markers between two groups. The levels of plasma FRAP as an indicator of antioxidant power was considerably increased after a month in both control and NOE-treated groups. We found that the NO content in the control group was decreased significantly 39.77 (31.43–55.03 vs 23.30 (17.79–25.88), P = 0.001). Additionally, the percent changes of NO in the NOE-treated group were higher than the control group. Plasma PCO and MDA levels after one month treating with NOE showed an insignificant decrease. However, the percent changes of these markers in intervention group increased considerably in comparison to placebo group. There was not any significant difference in the levels of antioxidant enzymes including SOD and CAT at the end of the study compared to the beginning of the study. In addition, the results showed that percent changes of SOD levels between the two groups were significant.

4. Discussion

Recently, several studies have reported the beneficial effects of using some herbal medicines to treat moderate to severe allergic asthmatic patients. Therefore, the treatment of asthma with herbal medicines has been considered as a form of complementary or alternative medicine (Lee et al., 2020; Uhm et al., 2012). In addition, some traditional herbs and herbal supplements are used in China to treat asthma (Lewis et al., 2021). In the present study, for the first time, the effect of NOE on plasma levels of oxidant and antioxidant factors, as well as inflammatory cytokines in patients with asthma, was evaluated. It was observed that treatment of the patients with NOE for four weeks led to a significant reduction in oxidative markers. Nevertheless, conflicting results were obtained regarding changes in serum levels of some inflammatory cytokines.

Asthma is one of the most common chronic inflammatory disorders that causes airway obstruction (Igde et al., 2018). The mechanisms involved in the airway obstruction are not fully understood. However, oxidative stress and inflammation have been shown to play a pivotal role in the pathogenesis of asthma. Chronic inflammation leads to the production of ROS (Nalban et al., 2019); consequently, the balance between oxidants and antioxidants will be disturbed, causing oxidative stress. The lungs are prone to oxidative damage because of their large surface area and abundant blood vessels. Due to their strong oxidative ability, ROS damage proteins, lipids, and DNA in cells, disrupting the function of these molecules (Karadogan et al., 2020; Nalban et al., 2019). Increased ROS leads to the infiltration of inflammatory cells into the lungs, stimulation of extracellular matrix protein production, and production of proinflammatory cytokines in airway epithelial cells (Huang et al., 2017). Several studies have shown significant increases in levels of oxidative stress biomarkers, including MDA, thiol oxidation, PCO, and exhaled nitric oxide (FeNO) in plasma, sputum, and bronchoalveolar lavage (BAL) fluid of asthmatic patients (Lewis et al., 2021). It has been suggested that natural antioxidants may play an effective role in improving the pathological symptoms of asthma by reducing oxidative stress in asthmatic mice (Huang et al., 2017). Our previous studies indicated that the NOE treatment for one month reduced oxidative stress markers in hemodialysis patients (Sedaghattalab et al., 2021). Furthermore, the antioxidant properties of NOE were demonstrated in animal models of cholestasis and acetaminophen-induced hepatotoxicity (Azarmehr et al., 2019; Sadeghi et al., 2019).

Direct measurement of free radicals is difficult due to their short half-lives. The harmful effects of ROS on biological molecules such as lipids and proteins have been well studied. Thus, oxidative stress is indirectly measured by examining its effects on biological molecules (Nalban et al., 2019). MDA is a product of lipid peroxidation and an indicator of oxidative damage caused by ROS (Sherif, 2018). In the present study,

Table 1
Baseline characteristics of the control and intervention groups.

Variable	Control	Intervention	*p* value
Age	56.44 ± 18.18	62.72 ± 15.28	0.280
Gender (female/male)	11/11	16/10	1
FRAP (μmol/L)	1067.75 (914.93–1191.50)	1164.62 (960.25–1278.06)	0.116
SOD (U/ml)	372.15 (355.60–377.92)	372.15 (355.68–380.52)	0.357
CAT (U/ml)	1.65 (0.52–3.64)	2.64 (1.49–4.41)	0.061
PCO (μmol/L)	8.65 (7.54–10.47)	9.08 (7.80–10.95)	0.444
NO metabolite (μmol/L)	39.77 (31.43–55.03)	37.79 (28.30–60.22)	0.950
TSH (μmol/L)	12.50 (11.67–14.30)	12.17 (10.17–14.19)	0.301
MDA (μmol/L)	1.96 (1.66–2.66)	2.05 (1.49–2.68)	0.820
IL-13 (pg/mL)	20.28 (18.85–23.38)	20.76 (18.85–21.83)	0.903
IgE (pg/mL)	73.51 (28.08–208.08)	181.37 (36.58–351.30)	0.196
TNF (pg/mL)	4.40 (4.09–5.01)	4.40 (4.09–4.70)	0.739
IL-1 (pg/mL)	1.06 (0.84–1.51)	0.84 (0.40–1.28)	0.052

Values are median (interquartile ranges) for data not normally distributed. FRAP: ferric reducing antioxidant power, SOD: superoxide dismutase, CAT: catalase, PCO: protein carbonyl, NO metabolite: nitric oxide metabolite, TSH: total thiol group, MDA: malondialdehyde, IL-13: interleukin-13, IgE: immunoglobulin E, TNF-α: tumor necrosis factor-α, IL-1: interleukin-1.

Table 2
Comparison of changes in the inflammatory markers during the study period between the control and intervention groups.

Variable	Baseline	After 1-months	*P* value	% change	*P* value
IL-13 (pg/mL)					
Control	20.28 (18.85–23.38)	21.71 (19.92–22.90)	0.022	−3.14 (−20.57, 34.28)	0.172
Intervention	20.76 (18.85–21.83)	20.04 (19.33–21.71)	0.048	−0.71 (−2.50, 0.71)	
IgE (pg/mL)					
Control	73.51 (28.08–208.08)	71.51 (38.65–241.51)	0.215	0.00 (−1.90, 2.73)	0.109
Intervention	181.37 (36.58–351.30)	211.65 (36.94–406.94)	0.528	8.42 (−6.64, 79.85)	
TNF-α (pg/mL)					
Control	4.40 (4.09–5.01)	4.16 (3.78–4.55)	0.446	0.38 (−0.15, 0.88)	0.886
Intervention	4.40 (4.09–4.70)	4.01 (3.90–4.32)	0.913	0.46 (−0.03, 0.80)	
IL-1 (pg/mL)					
Control	1.06 (0.84–1.51)	0.84 (0.40–1.28)	0.184	0.22 (−0.22, 0.83)	0.003
Intervention	0.84 (0.40–1.28)	1.28 (0.62–1.95)	0.029	−0.44 (−0.94, 0.22)	

Values are median (interquartile ranges) for data not normally distributed. IL-13: interleukin-13, IgE: immunoglobulin E, TNF-α: tumor necrosis factor-α, IL-1: interleukin-1.

Table 3
Comparison of differences in oxidative stress parameters during the study between the control and intervention groups.

Variable	Baseline	After 1-months	*P* value	% change	*P* value
FRAP (μmol/L)					
Control	1067.75 (914.93–1191.50)	1228.37 (1061.18–1472.43)	0.015	211.25 (60.62, 272.18)	0.049
Intervention	1164.62 (960.25–1278.06)	1573.37 (1390.87–1715.25)	0.000	442.50 (157.50, 643.75)	
SOD (U/ml)					
Control	372.15 (355.60–377.92)	297.80 (291.63–314.99)	0.468	64.83 (54.54, 72.06)	0.000
Intervention	372.15 (355.68–380.52)	287.01 (278.02–295.77)	0.575	86.50 (82.80, 91.95)	
CAT (U/ml)					
Control	1.65 (0.52–3.64)	0.81 (0.28–1.56)	0.892	1.31 (−0.75, 2.61)	0.605
Intervention	2.64 (1.49–4.41)	1.31 (0.62–2.29)	0.631	1.44 (0.47, 2.52)	
PCO (μmol/L)					
Control	8.65 (7.54–10.47)	8.47 (6.32–9.95)	0.333	−0.89 (−1.95, 1.18)	0.000
Intervention	9.08 (7.80–10.95)	5.60 (4.45–6.54)	0.701	−3.81 (−5.11, −1.88)	
NO metabolite (μmol/L)					
Control	39.77 (31.43–55.03)	23.30 (17.79–25.88)	0.001	−16.25 (−37.83, −2.38)	0.048
Intervention	37.79 (28.30–60.22)	8.89 (6.50–15.58)	0.232	−30.14 (−47.86, −15.40)	
TSH (μmol/L)					
Control	12.50 (11.67–14.30)	15.25 (11.87–18.40)	0.376	2.02 (−3.02, 5.62)	0.488
Intervention	12.17 (10.17–14.19)	12.82 (9.59–16.08)	0.329	0.69 (−2.73, 3.69)	
MDA (μmol/L)					
Control	1.96 (1.66–2.66)	2.16 (1.55–2.73)	0.694	−0.12 (−0.47, 0.83)	0.010
Intervention	2.05 (1.49–2.68)	1.58 (1.13–1.88)	0.555	−0.64 (−1.14, −0.16)	

Values are median (interquartile ranges) for data not normally distributed. FRAP: ferric reducing antioxidant power, SOD: superoxide dismutase, CAT: catalase, PCO: protein carbonyl, NO metabolite: nitric oxide metabolite, TSH: total thiol group, MDA: malondialdehyde.

MDA levels in both placebo and intervention groups decreased insignificantly at the end of the study, although the percent changes of MDA in NOE treated patients was dramatically higher than the placebo group. These results were consistent with our previous investigation, which showed that NOE reduced MDA in hemodialysis patients (Sedaghattalab et al., 2021). The reduction in MDA content may be due to the protective effects of antioxidant molecules in the extract against cell membrane injury caused by free radicals. Antioxidant activity of NOE has been demonstrated by preventing lipid peroxidation and free radicals scavenging (Clemente et al., 2021).

Among ROS-derived modifications, PCO is considered as a major marker of oxidative stress in various cells and tissues. Misso et al. showed in a study that plasma PCO in asthmatic patients increased significantly compared to the control group (Misso and Thompson, 2005). In this work, we found that PCO levels were reduced in the intervention group. As a matter of fact, the percent changes of PCO in the NOE treated group were higher than in the placebo group, which can illustrate the antioxidant properties of NOE. In accordance with the current study, NOE improved oxidative stress in chronic hemodialysis patients by reducing PCO levels (Sedaghattalab et al., 2021).

Although the exact mechanism of increase in NO metabolite levels and its role in the pathogenesis of asthma has not been well studied, it is suggested that NO may cause the production of active nitrogen species and subsequently induces lung inflammation. Detrimental effects of NO have been attributed to peroxynitrite resulting from its reaction with superoxide (Misso and Thompson, 2005). Following the conversion of NO to peroxynitrite, its sources are depleted and the signaling pathway related to dilation and relaxation of bronchial smooth muscle is disrupted (Bowler, 2004). Several studies have shown increased NO levels in patients with asthma as well as laboratory models of this disorder (Khaldi et al., 2018; Nadeem et al., 2003). In the present study, at the end of the treatment period in both intervention and placebo groups, NO levels decreased compared to the beginning of the study, although the percent changes of NO were higher in the NOE-treated group. These results, which are consistent with our previous studies, can indicate the effect of NOE on reducing oxidative stress and lung inflammation (Sedaghattalab et al., 2021).

FRAP assay is considered to be one of the most suitable methods for determination the antioxidant capacity of various biological fluids such as plasma, serum, and BAL fluids (Emin et al., 2010). Yadav et al. Showed that FRAP levels were significantly lower in asthmatics compared to healthy individuals (Yadav and Saini, 2016). Treatment of patients with NOE resulted in a marked increase in plasma FRAP levels. In the previous investigation, we found that total antioxidant capacity (TAC) levels increased after one month treatment with NOE in chronic hemodialysis patients (Sedaghattalab et al., 2021), which was in line with the present study.

Some studies have shown alterations in the levels of various antioxidants in asthmatic patients that may increase or decrease, depending on whether the cell responds defensively or the oxidants overcome the antioxidant system (Nadeem et al., 2003). GPX and SOD are regarded to be the main antioxidants in the lungs and their activity is reduced in asthma (Rajizadeh et al., 2019). Contrary to our previous findings (Sedaghattalab et al., 2021), in the present study, SOD decreased after one month treatment with NOE compared to the beginning of the study. It is noteworthy that the levels of this enzyme also decreased in the placebo group. Therefore, SOD reduction in the intervention group may not be associated with NOE administration.

Cytokines contribute to asthma by activating and surviving inflammatory cells. Proinflammatory cytokines including TNF-α and IL-1β play an important role in organizing airway inflammatory responses (Rajizadeh et al., 2019). In asthma, the immune response is induced by Th2 cells, which is associated with the infiltration and activation of leukocytes in the airways and the overproduction of cytokines, including IL-4, IL-5, and IL-13 (Charrad et al., 2016). In the present study, treatment with NOE for four weeks reduced IL-13 cytokines significantly, while IL-1 levels increased at the end of the treatment period compared to the beginning of the study. Sadeghi et al. Showed the anti-inflammatory properties of NOE in different models of inflammation, which was not consistent with this study (Sadeghi et al., 2014).

5. Conclusion

In summary, in the current study, four weeks treatment with NOE represented that this plant can be effective in improving oxidative stress in patients with asthma by reducing oxidative markers and increasing total antioxidant capacity. However, the results regarding antioxidant enzymes and some inflammatory factors were not aligned with other studies. Therefore, in order to evaluate the possible therapeutic effect of NOE in asthma, more clinical trials with different methods are needed.

Funding

This research was funded by Yasuj University of Medical Sciences, grant number 990127.

CRediT authorship contribution statement

Nasrin Shakerinasab: Conceptualization, Investigation, Writing – original draft. **Javad Mottaghipisheh:** Software, Validation, Formal analysis, Writing – review & editing. **Mahdieh Eftekhari:** Methodology, Writing – review & editing. **Hossein Sadeghi:** Data curation. **Fatemeh Bazarganipour:** Visualization. **Reza Abbasi:** Conceptualization, Methodology, Resources, Supervision. **Amir Hossein Doustimotlagh:** Conceptualization, Methodology, Validation, Resources, Writing – original draft, Supervision, Funding acquisition. **Marcello Iriti:** Validation, Resources, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments:

The authors wish to thank Dr. Arash Asfaram for the preparation of *Nasturtium officinale* plant.

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