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A double-blind, randomized, placebo-controlled clinical trial on the effect of carvacrol on serum cytokine levels and pulmonary function tests in sulfur mustard induced lung injury

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ABSTRACT

Objective: The aim of this study was to evaluate the effect of carvacrol on serum levels of interleukins (IL-2, IL-4, IL-6, IL-8, and IL-10), interferon-gamma (IFNγ) levels and pulmonary function tests (PFT) in patients who were exposed to sulfur mustard (SM).

Methods: Twenty patients exposed to SM 27–30 years ago were divided to placebo and carvacrol (1.2 mg/kg/day) treated groups (n = 10 for each group). Drugs were given in a double-blind manner for two months. Serum levels of cytokines and PFT values including; maximum mid-expiratory flow (MMEF) and maximum expiratory flow at 25, 50 and 75% of vital capacity (MEF25, 50 and 75) were measured at the beginning (step 0), one and two month (steps I and II, respectively) after starting the treatment.

Results: The serum levels of IL-2, IL-4, IL-6 and IL-8 were significantly decreased in step I and II compared to step 0 ($p < 0.05$ to $p < 0.001$), while the serum levels of IL-10 and IFNγ were increased in step II compared to step 0 ($p < 0.01$, for both cases) and IFN-γ/IL-4 ratio was enhanced in step II compared to step 0 ($p < 0.001$) in carvacrol treated group. MMEF, MEF75, and 50 values were significant increase in step I and II compared to step 0 ($p < 0.05$ to $p < 0.001$) in carvacrol treated group.

Conclusion: Treatment with carvacrol for two months reduced inflammatory cytokine, while increased anti-inflammatory cytokines and improved PFT tests in SM induced lung injury.

1. Introduction

Sulfur mustard (SM) is a vesicant agent which lead to short and long term injury in different organs including; Lung, eyes, skin, heart, nervous and digestive systems [1,2]. SM injured too many Iranians veterans (more than 100,000) which many of them are still suffering from the effects of exposure to this agent [3]. Basic and molecular mechanisms involved in SM clinical symptoms are unclear and clinical management strategies for treatment of injured patients are not well defined [4]. Some studies indicating important alterations in immunological parameters including TCD4+, TCD8+, Natural Killer cells, immunoglobulins and cytokines regarding to immunological status of long term effect of SM exposure [5,6].

SM exposed in guinea pig caused accumulation of inflammatory cell in the airways and lung which lead to structural and functional alterations in the respiratory tract [7]. SM exposure also increased inflammatory cytokines including tumor necrosis factor-α (TNFα), IL-1α, IL-1β, and reactive oxygen radicals. In addition, SM in the lung also can cause serious pathological changes including airway inflammation, parenchymal tissue destruction and airway obstruction which can lead to asthma or chronic obstructive pulmonary disease (COPD) [8]. Carvacrol is a monoterpenic phenol and the main component of essential oil in various plants especially *Origanum vulgare* (oregano) and Thymus genus from Lamiaceae family [9].

Abbreviations: IL-, Interleukin; IFNγ, Interferon-gamma; PFT, Pulmonary function tests; SM, Sulfur mustard; MMEF, Maximum mid-expiratory flow; MEF25, 50 and 75, maximum expiratory flow at 25, 50 and 75% of vital capacity; TNFα, Tumor necrosis factor-α; COPD, Chronic obstructive pulmonary disease; Oregano, *Origanum vulgare*; WBC, White blood cell; MDA, Malondialdehyde

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Carvacrol is allowed for food use by the FDA and was involved in the list of chemical flavorings by the Council of Europe [10]. Several pharmacological properties including, antioxidant [11], anti-inflammatory and immunomodulatory [12,13], antitumor [14] and antimicrobial [15] effects were shown for carvacrol in previous studies. Carvacrol decreased inflammation by various mechanisms such as; inhibition of cyclooxygenase-2 and subsequently decreased prostaglandin E2 production [16], reduced production of inflammatory mediators including TNF-α, IL-6, iNOS and IL-10 [17–19], and also reduced IL-4, endothelin, IgE and eosinophil peroxidase levels while increased IFN-γ in the serum of treatment sensitized guinea-pigs [20,21]. Total white blood cell (WBC), eosinophil, serum levels of IL-8 and malondialdehyde (MDA) were also decreased in animal model of chronic obstructive pulmonary disease (COPD) treated with carvacrol [22].

Therefore, the aim of this study was to examine the preventive therapeutic effect of two month treatment with carvacrol on the serum levels of inflammatory and anti-inflammatory cytokines and pulmonary function tests (MMEF and MEF25, 50 and 75) in patients with lung disorder due to SM exposure, 27–30 years ago.

2. Materials and methods

2.1. Participants

Thirty two eligible male patients were selected on Aug 2016 which seven of them were declination to participations the study or not fulfilling the inclusion criteria. Twenty five chemical war victims were enrolled and randomly allocated to two groups by simple randomization which five of them did not continued the study and 20 subjects completed the study on Mar 2017 (Fig. 1). Exposure to SM is the eligibility criteria for participants. Ages of participants in the placebo and carvacrol treated groups were 55.20 ± 7.35 and 57.30 ± 7.34 (mean ± SD) years, and their heights were 172.30 ± 7.34 and 170.40 ± 5.21. All patients were referred by an organization for chemical war victims in Iran. The distance between the exposed veterans and the bomb site ranged from 500 to more than 3000 m and the time from the exposure to SM ranged from 27 to 30 years. All studied subjects were nonsmokers; none of them reported a recent respiratory infection or had a history of asthma or chronic obstructive pulmonary disease (COPD) prior to exposure to chemical warfare. All subjects also have no history of medications for cardiac, high blood pressure and diabetes. Demographic information of participants was presented in Table 1.

This clinical trial was approved by the Ethical Committee of the Mashhad University of Medical Sciences (Code: 920537), and all subjects signed informed consent. It was also registered in Iranian Registry of Clinical Trials (IRCT Code: IRCT2014031617020N1).

2.2. Preparation of carvacrol drug

Carvacrol pharmaceutical grade (90%) was purchased from Ji'AnHaiRui Natural Plant Co. (China). Pellets were produced by coating of carvacrol onto the non-pareil beads (850–1180 μm) using fluidized bed coater (Wurster insert, Werner Glatt, Germany). 80% (w/v) of carvacrol was prepared by dispersing of 5% HPMC and 2% Talc in absolute ethanol. This suspension was passed through a 140 mesh sieve. The suspension was sprayed onto non-pareils using fluidized bed coater. The suspension was stirred throughout layering process. The carvacrol layering process was carried out to produce pellets with about 7.5 and 11.75% (w/w) carvacrol load. After coating, the pellets were re-coated with HPMC 5% solution and fluidized for about 5 min and then were kept in an oven for 2 h at 40 °C. The amount of carvacrol in pellets was measured by GC method. The GC analysis was performed using a Varian CP-3800 equipped with FID detector, fused-silica column (CP-Sil 8CB, 50 m × 0.25 mm, film thickness 0.12 μm).

Although all drugs were used during 2 months, an accelerated stability studies (40 °C ± 2 °C/75% RH ± 5% RH)1 were done for a period of 6 months. The results displayed that no significant changes were observed through this period [23]. Drugs including, carvacrol and placebo were labeled by drug packager.

2.3. Treatment groups

Twenty chemical war victims were divided by the first author to two groups used from simple randomization included: placebo group (P) and treatment group with carvacrol (1.2 mg/kg/day) three times a day (n = 10 for each group). Drugs were prescribed in double-blind manner for two months according to the previous studies [20,22,37]. All patients were advised to continue their pervious treatment regimen as described in Table 1. Blood sampling in not fasting situation were carried out in three steps during trial including; pretreatment (step 0), one month after treatment (step I) and two months after treatment (step II). The enrollment flow chart of SM induced lung injury was showed in Fig. 1.

2.4. Serum collection and cytokine measurement

Peripheral blood samples in non-fasting condition were collected from each patient, allowed to clot at room temperature and centrifuged for 20 min at 2000*g*. Serum was removed, aliquoted and stored at −80 °C until future measurements.

The concentrations of serum cytokines, including IL-2, IL-4, IL-6, IL-8, IL-10 and IFN-γ were measured using the EV3513 cytokine biochip array (Randox Laboratories, London, UK) and competitive chemiluminescence immunoassays (Randox Laboratories, London, UK), according to the manufacturer's instructions [24], using the Randox Evidence Investigator. The Evidence Investigator is an automated analyser for the simultaneous detection of multiple cytokines from a single sample and uses sandwich chemiluminescent methods [24].

2.5. Measurement of pulmonary function tests (PFT)

PFE test including; maximum mid-expiratory flow (MMEF), and maximum expiratory flow rate (MEF25, 50 and 75) were measured using a spirometer with a pneumotachograph sensor (Model ST90, Fukuda, Sangyo Co., Ltd., Japan). MMEF and MEF25, 50 and 75 were measured based on standards outlined by the American Thoracic Society (ATS). Prior to measurement of PFT, measurement technique was demonstrated by the operator, and PFT was measured in a sitting position and wearing nose clips. PFT measurement was performed two or three times and the best values were chosen among measurements.

2.6. Statistical analysis

The data of different cytokines and pulmonary function test were expressed as mean ± SD. The percent change of each variable during treatment period was calculated using the following equation:

$$\text{I or II}/0 = \frac{(\text{value in step I or II} - \text{value in step 0}) \times 100}{\text{value in step 0}}$$

$$\text{I/II} = \frac{(\text{value in step II} - \text{value in step I}) \times 100}{\text{value in step I}}$$

The comparisons of data in each group during three steps of the study were analyzed using repeated measures procedure. Pairwise comparisons were performed by Bonferroni test. The comparison of PFT changes during treatment period between treated and placebo groups were analyzed by independent samples test. p-value < 0.05 was considered to be statistically significant.

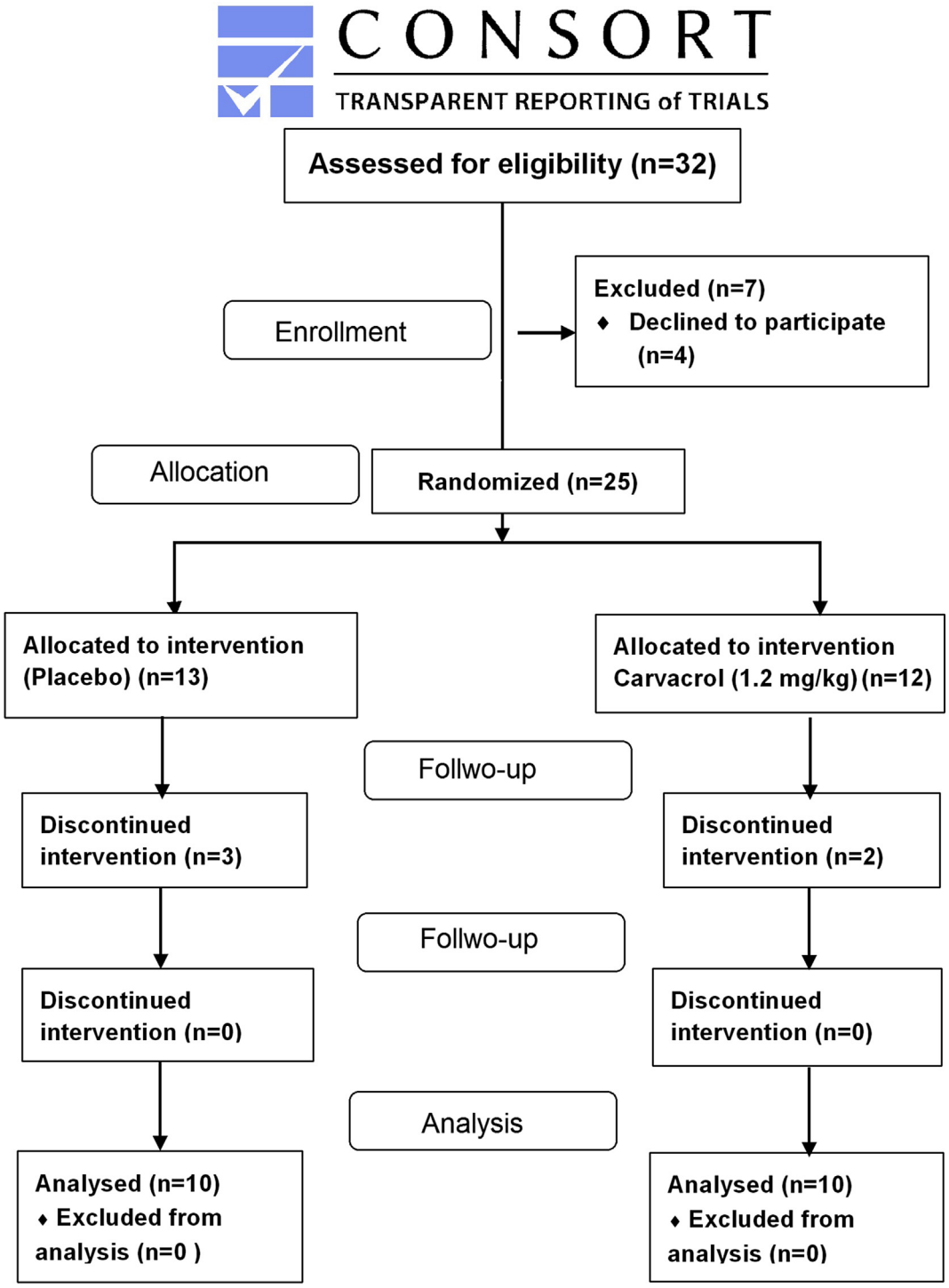

Fig. 1. Flowchart of the study selection of patients.

Table 1
Demographic information of SM exposed patients, participated in the study.

Group	Age	Height	Wight	BMI	n
Placebo	55.20 ± 7.35	172.30 ± 8.61	79.50 ± 3.35	27.87 ± 1.02	10
Carvacrol (1.2 mg/kg)	57.30 ± 7.34	170.40 ± 5.21	76.10 ± 4.35	25.97 ± 1.10	10
Previous treatment regimen	Respiratory	inhaled salbutamol and corticosteroid, oral corticosteroid, theophylline in 10 placebo and 10 carvacrol treated patients			
	Others	Statins, vitamin supplementation, metformin, losartan in 4 placebo and 7 carvacrol treated patients			

SM: Sulfur mustard; BMI: Body Mass Index.

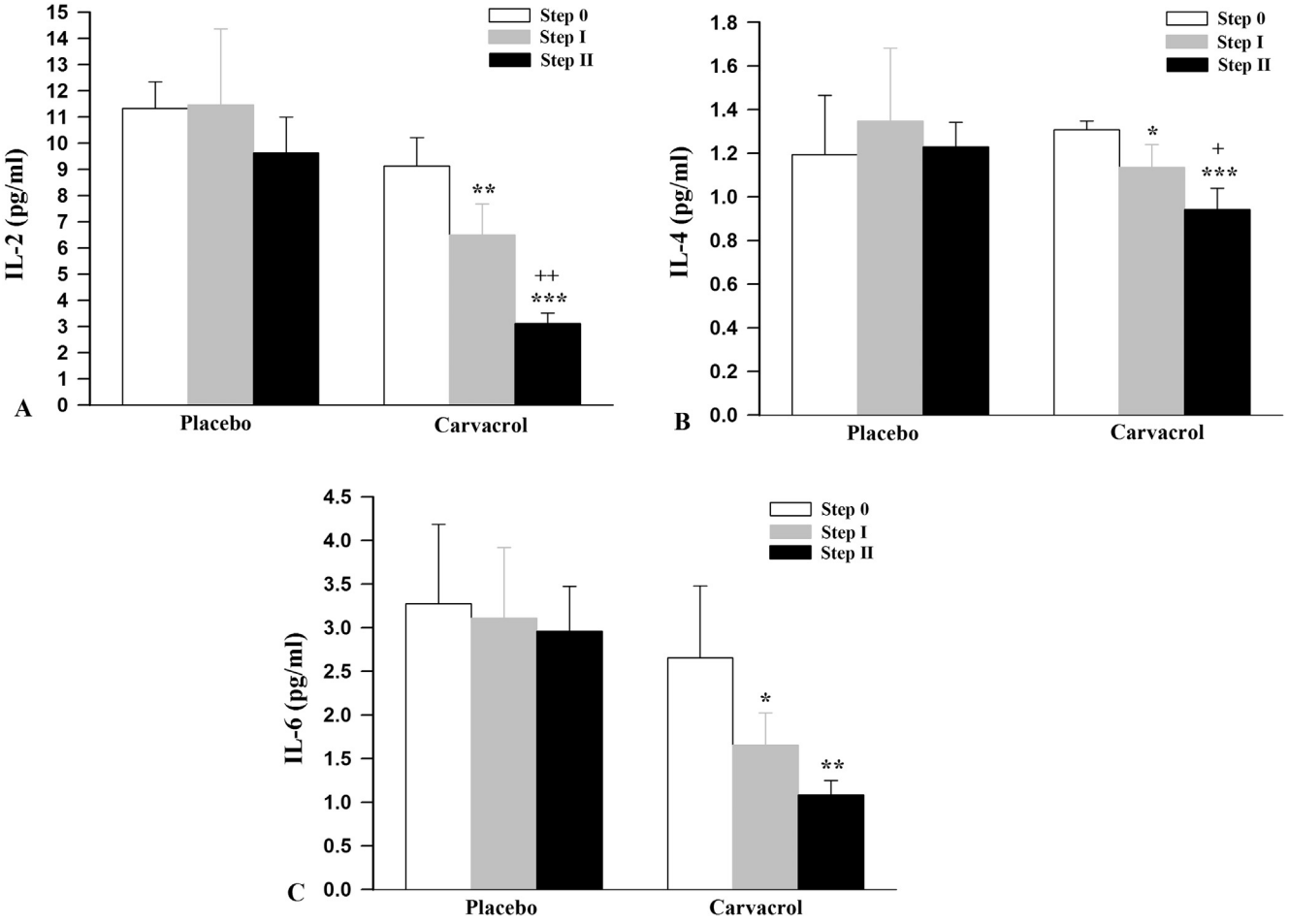

Fig. 2. Serum levels of IL-2 (A), IL-4 (B), and IL-6 (C) at the baseline (step 0), the end of first (step I), and second (step II) month of the study in treated groups with placebo and carvacrol (1.2 mg/kg). Data were presented as mean ± SD. Significant difference in values of the step I and II compared to step 0, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Significant difference in the values of step II compared to step I, $^{+}p < 0.05$, $^{++}p < 0.01$. Statistical comparisons were performed using repeated measures procedure. Pairwise comparisons were performed by Bonferroni test.

3. Results

3.1. The effects of carvacrol on serum cytokines

The serum levels of IL-2 and IL-4 were significantly decreased in step I ($p < 0.01$ and $p < 0.05$, respectively), and in step II ($p < 0.001$ for both cases), compared to the step 0 in carvacrol treated group. In addition the levels of IL-2 and IL-4 in step II were significantly reduced compared to step I ($p < 0.01$ and $p < 0.05$, respectively, Fig. 2A and B). The serum levels of IL-6 was also significantly decreased in step I and II compared to the step 0 ($p < 0.05$ and $p < 0.01$, respectively) in carvacrol treated group (Fig. 2C).

The serum level of IL-8 was significantly decreased in step I and II compared to the step 0 ($p < 0.01$ and $p < 0.001$, respectively) (Fig. 3A), while the serum levels of IL-10 and IFN-γ were significantly increased in carvacrol treated groups, in step II compared to step 0 ($p < 0.01$, for both cases, Fig. 3B and C). In addition, the serum level of IL-10 was significantly increased in step II compared to step I ($p < 0.01$, Fig. 3B). Carvacrol treatment was also lead to enhancement of the IFN-γ/IL-4 ratio (Th_1/Th_2 balance) in step II compared to step 0 ($p < 0.001$). In addition, the IFN-γ/IL-4 ratio was significantly increased in step II compared to step I ($p < 0.05$, Fig. 3D). Cytokines levels in the placebo group were not significantly changed in step I and II compared to step 0.

The percent change of IL-2 and IL-6 in carvacrol treated group ($p < 0.05$ for both cases) and the level of IL-10 in step I relative to step 0 were significantly Improved compared to placebo group ($p < 0.05$, Fig. 4A). The percent change of IL-2, IL-4, IL-6 and IL-8 in carvacrol treated group ($p < 0.05$ to $p < 0.01$), as well as the percent change of IL-10 and IFN-γ in step II relative to step 0 were significantly improved compared to placebo group ($p < 0.001$ and $p < 0.01$, respectively Fig. 4B). The percent change of IL-2 and IL-6 in carvacrol treated group ($p < 0.05$ and $p < 0.001$, respectively), and the percent change of IL-10 in step II relative to step I were significantly improved compared to placebo group ($p < 0.05$ Fig. 4C).

3.2. The effects of carvacrol on PFT

PFT values in the placebo group were not significantly changes in step I and II compared to step 0 (Fig. 5A). MMEF, MEF75 and 50 values were significant increase in carvacrol treated group in step I and II compared to step 0 ($p < 0.05$ to $p < 0.001$, Fig. 5B). In addition, MMEF and MEF75 values were significant increase in step II compared to step I ($p < 0.05$ for both cases, Fig. 5B).

The percent change of MEF50 in carvacrol treated group, in step I relative to step 0 (I/0) ($p < 0.01$, Fig. 6A), and also MEF50 and MEF25 in step II relative to step 0 (I/0) ($p < 0.01$ and $p < 0.05$, respectively, Fig. 6B) were significantly improved compared to placebo group. In addition, the percent change of MEF75 and 50, in step II relative to step I (II/I) were significantly improved compared to placebo group ($p < 0.01$ and $p < 0.05$, respectively, Fig. 6C).

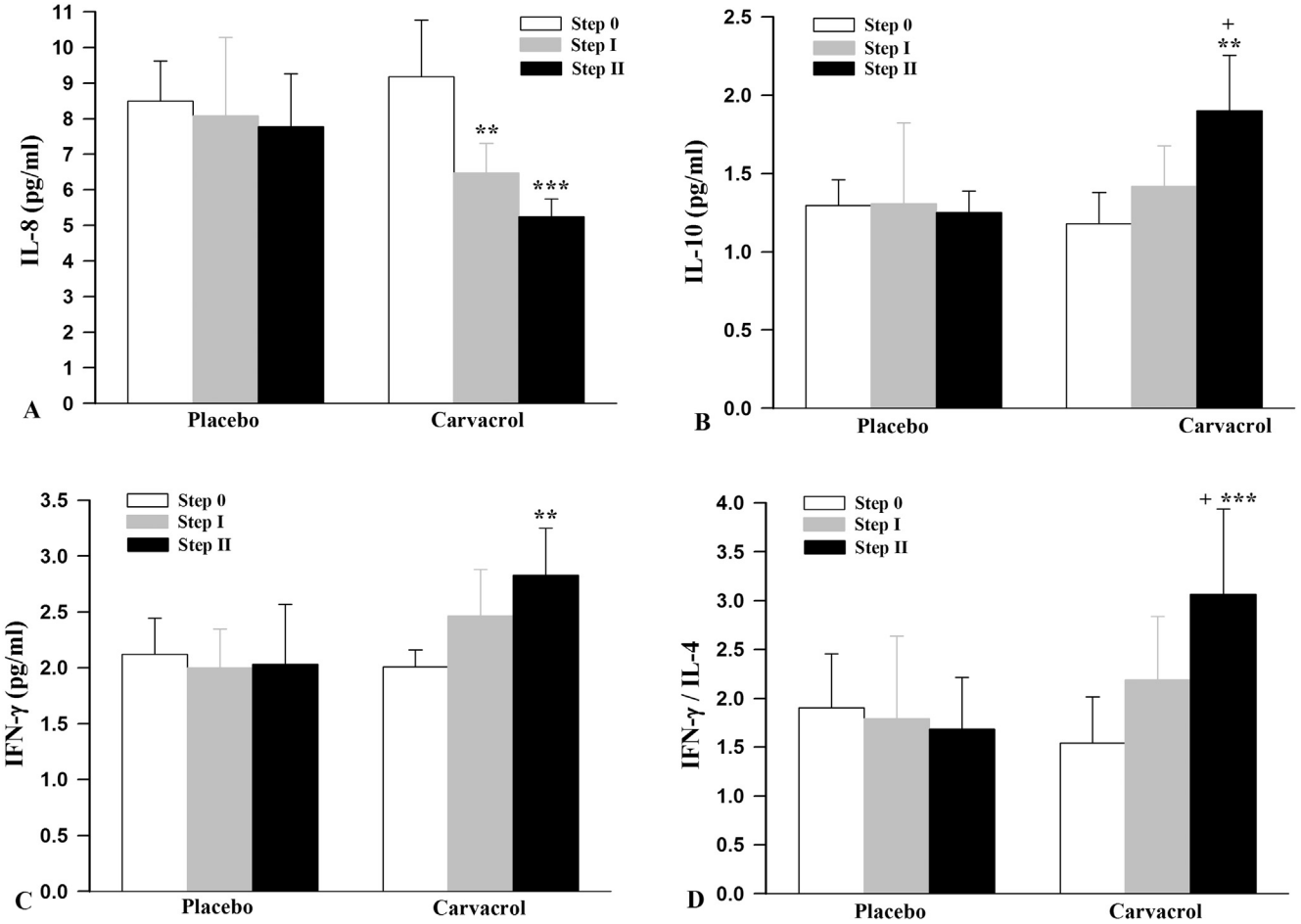

Fig. 3. Serum levels of IL-8 (A), IL-10 (B), IFN-γ (C) and IFN-γ/IL-4 ratio (Th_1 to Th_2 balance) (D) at the baseline (step 0), the end of first (step I), and second (step II) month of the study in treated groups with placebo and carvacrol (1.2 mg/kg). Data were presented as mean ± SD. Significant difference in values of the step I and II compared to step 0, $^{**}p < 0.01$, $^{***}p < 0.001$. Significant difference in the values of step II compared to step I, $^{+}p < 0.05$. Statistical comparisons were performed using repeated measures procedure. Pairwise comparisons were performed by Bonferroni test.

4. Discussion

In the current study the effect of two months treatment with carvacrol (1.2 mg/kg/day) on serum levels of cytokines and PFT (MMEF and MEF25, 50 and 75) values in patients with lung disorder due to exposure to SM about 30 years ago were assessed. According to the results of this study, carvacrol significantly decreased inflammatory cytokines including, IL-2, IL-4, IL-6 and IL-8 during two months treatment period. Moreover, carvacrol treatment significantly increased the levels of anti-inflammatory cytokines, IL-10, IFN-γ and IFN-γ/IL-4 ratio at the end of one and two months treatment compared to baseline values.

Inhalation of SM induces an acute pro inflammatory response in the lung and induced the expression of pro-inflammatory cytokines and chemokines in the lung tissues and bronchoalveolar lavage (BAL) of SM-exposed rats. Additionally, SM exposure significantly increased the expression of pro-inflammatory cytokines including (IL-1β, TNF-α, IL-2, and IL-6) in the lung [25]. The serum level of IL-6 was increased in patients with SM poisoning and stable COPD, which indicated a direct association with airflow limitation [26]. In an *in vitro* study, the levels of IL-8 was significantly increased in human lung small airway cell (SAC) exposed to SM [27]. Increased IL-6 and decreased IFN-γ in BAL fluid and serum of exposed rat to SM were also reported [28]. Serum levels of interleukin IL-8, IL-6 and cell adhesion molecules (e.g. selectins) were also increased in SM-exposed subjects, 20 years after exposure [29,30]. All described studies indicated cytokines changes due to SM exposure.

It was shown that D-galactosamine (D-GalN)-induced hepatotoxic in rats significantly up-regulated mRNA and protein expressions of TNF-α, IL-6, iNOS, cyclooxygenase-2 (COX-2), NF-κB and the expressions of these genes were significantly down-regulated by treatment with carvacrol [17]. Treatment of sensitized animals with carvacrol significantly decreased IL-4 and endothelin level but significantly increased IFN-γ levels [20]. The antihyperlipidemic and anti-inflammatory effect of combination therapy of carvacrol and rosiglitazone on diabetic mice showed that the alteration of total cholesterol, triglycerides, phospholipids and free fatty acids in plasma and liver tissue for inflammatory cytokines (TNF-α and IL-6) significantly modulates towards normality [31]. Administration of carvacrol attenuated the paw edema and reduced the IL-1β, prostaglandin E2, COX-2 and reduced IL-1β mRNA expression. Furthermore, the levels of IL-10, an anti-inflammatory cytokine, and the IL-10 mRNA expression in the inflamed paw were enhanced by carvacrol [32]. The results of the present study showed increased IFN-γ/IL-4 ratio which indicate increased Th1/Th2 ratio due to carvacrol treatment in patients with lung disorder due to SM exposure. In the previous study carvacrol significantly increased IFN-γ and FOXP3 genes expression but significantly decreased IL-4, TGF-β and IL-17 genes expression in splenocytes from sensitized animals. Furthermore, carvacrol significantly increased IFN-γ/IL-4 ratio in the treated splenocytes compared to untreated cells [33]. In guinea pig model of asthma also carvacrol treatment showed increased IFN-γ/IL-4 ratio [20] which support the findings of the present study.

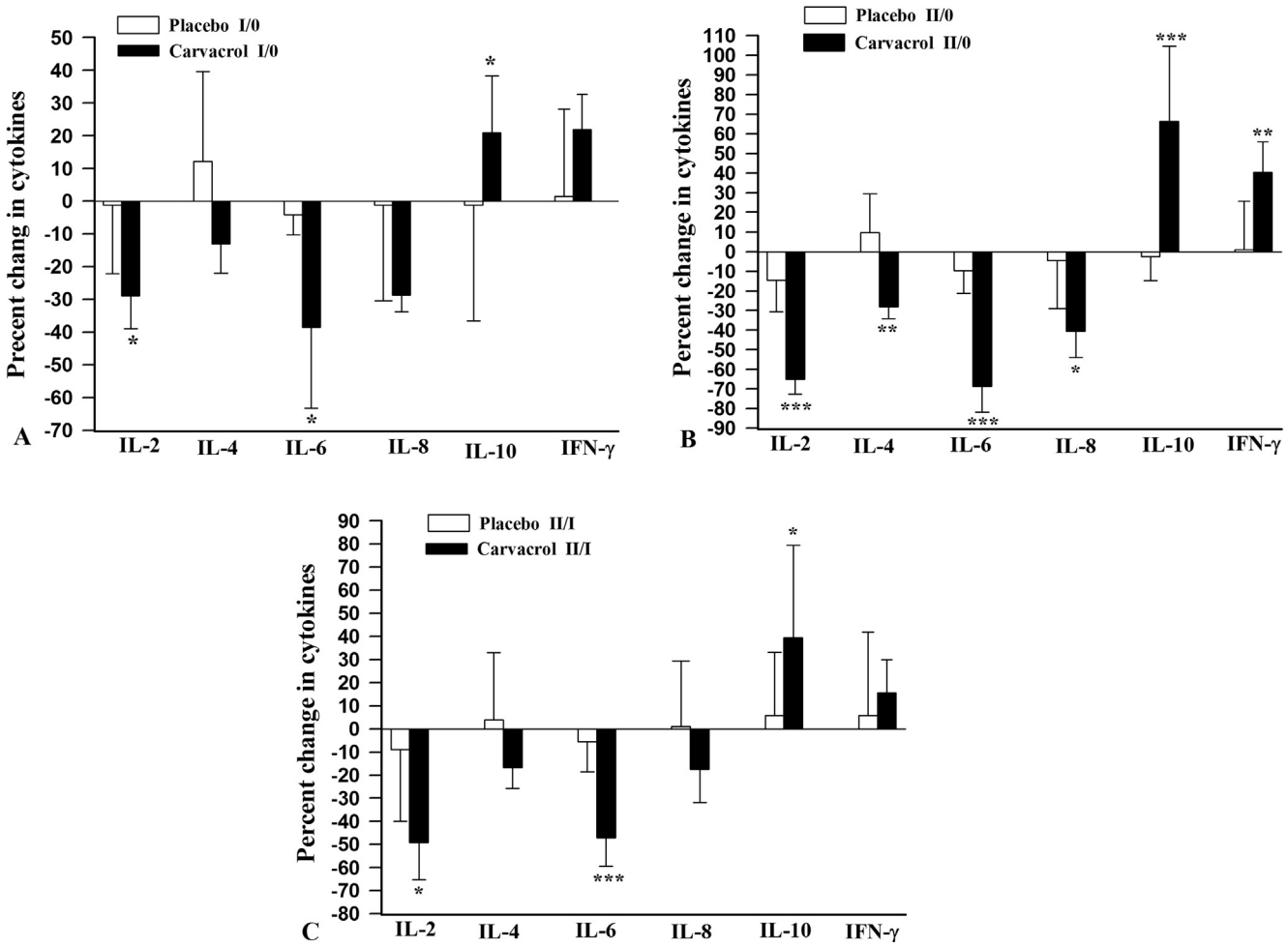

Fig. 4. Percent change in serum cytokines in treated groups with placebo and carvacrol (1.2 mg/kg) treated groups in step I relative to step 0 (A), step II relative to step 0 (B) and step II relative to step I (C). Data were presented as mean ± SD. Significant difference in the treated groups compared to the placebo group, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.01$. Statistical comparisons were performed using independent *T*-test.

All the above studies indicate a potent anti-inflammatory property for carvacrol and support the findings of the present study. The improvement effect of carvacrol on inflammatory and anti-inflammatory cytokines and the absence of these effects in the placebo treated group indicate potential preventive therapeutic effect of carvacrol in inflammatory lung diseases including the lung inflammation in SM exposed patients.

The results of the present study also showed that one and two month

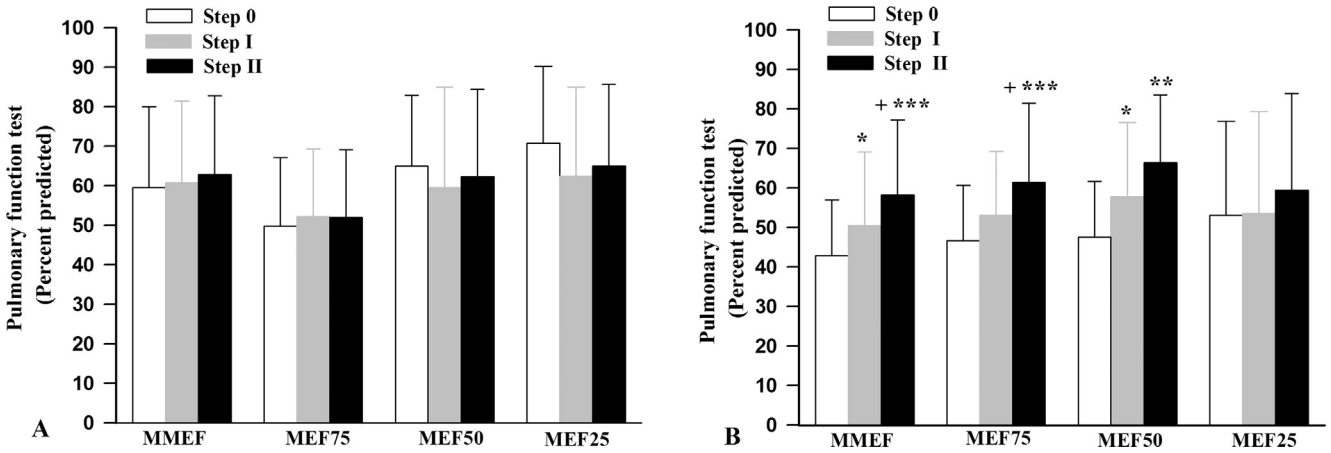

Fig. 5. Maximum mid-expiratory flow (MMEF), and maximum expiratory flow rate at 25, 50 and 75% of vital capacity (MEF25, MEF50 and MEF75 respectively) in treated groups with A: placebo group and B: carvacrol (1.2 mg/kg) at the baseline (step 0), the end of first (step I), and second (step II) month of the study. Data were presented as mean ± SD. Significant difference in the step I and II compared to step 0; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Significant difference in step II compared to step I; $^{+}p < 0.05$. Statistical comparisons were performed using repeated measures procedure, with time as within-subjects. Pairwise comparisons were performed by Bonferroni test.

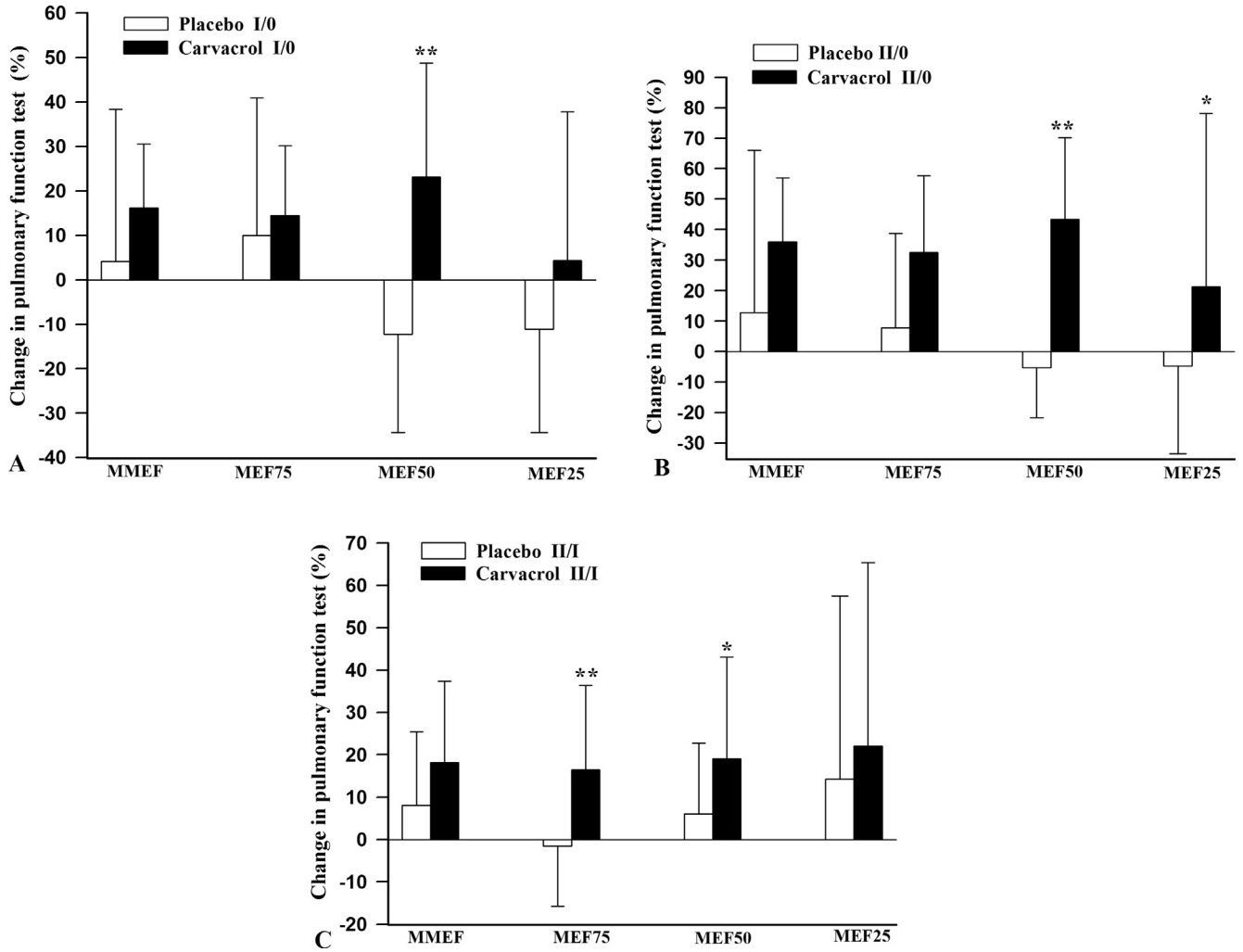

Fig. 6. Percent change in maximal mid-expiratory flow (MMEF), and maximum expiratory flow rate at 25, 50 and 75% of vital capacity (MEF25, MEF50 and MEF75 respectively) in placebo and carvacrol treated groups (1.2 mg/kg) in step I relative to step 0 (A), step II relative to step 0 (B) and step II relative to step I (C). Data were presented as mean ± SD. Significant difference in the treated groups compared to the placebo group, $^{*}p < 0.05$, $^{**}p < 0.01$. Statistical comparisons were performed using independent *T*-test.

treatment with carvacrol resulted in increased MMEF, MEF75 and 50 values. The percent improvement in MEF25, MEF50 and MEF75 values in carvacrol treatment group were significantly higher compared to placebo treatment group. Therefore, the results of the present study, suggested a potential preventive therapeutic effect of carvacrol in lung disorders of SM exposed patients.

The results of previous studies also shown that some of the PFT parameters including FVC, MMEF, and MEF75 significantly increased due to inhaled salbutamol in SM-exposed patients compared to control group 18–20 years after exposure [34]. SM exposure also caused chronic bronchitis, pulmonary fibrosis, and bronchiectasis, in SM-induced patients 10 years after exposure [35,36]. The results of previous study showed that treatment with carvacrol in a 2 month study period increased pulmonary function tests but decreased respiratory symptoms and inflammation in asthmatic patients [37]. Furthermore, the relaxant effect of carvacrol on tracheal smooth muscle of guinea pigs [38,39] and its inhibitory effect on muscarinic [40] and histamine H_1 receptors [41] as well as stimulatory effect on β2-adrenoreceptors in tracheal smooth muscle were shown [42]. The relaxant effect of carvacrol on airway smooth muscle (bronchodilatory effect) supports the results of present study. The chest high resolution CT scans and pulse oximetry were not carried out in the treatment with carvacrol and placebo groups which should be examined in further studies. The treatment period of the current study was chosen according to our previous study, indicating a two months treatment with carvacrol revealed significant therapeutic effects in asthmatic patients [37]. However, studying the therapeutic effect of carvacrol in patients with lung disorder due to exposure to SM for a longer treatment period is encouraged. The major limitation of the present study is the numbers studied patients. In fact, there are limited SM exposed patients available which some of them suffer from severe respiratory disease and other complications due to SM exposure. In addition, in this study more patients were recruited but some of them declined to participate the study and some others did not continue the study.

In conclusion, the results of the present study suggested the possible therapeutic effect of carvacrol on lung disorders of SM exposed individuals by reduction of inflammatory cytokines and increased anti-inflammatory cytokines and also improvement of PFT values including MMEF, MEF25, MEF50 and MEF75.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] S. Somani, S. Babu, Toxicodynamics of sulfur mustard, Int. J. Clin. Pharmacol. Therap. Toxicol. 27 (1989) 419–435.
[2] D. Evison, D. Hinsley, P. Rice, Regular review: chemical weapons, BMJ: Br. Med. J. 324 (2002) 332.
[3] K. Ghabili, P.S. Agutter, M. Ghanei, K. Ansarin, M.M. Shoja, Mustard gas toxicity: the acute and chronic pathological effects, J. Appl. Toxicol. 30 (2010) 627–643.
[4] M. Balali-Mood, M. Hefazi, Comparison of early and late toxic effects of sulfur mustard in Iranian veterans, Basic. Clin Pharmacol Toxicol. 99 (2006) 273–282.
[5] Z.M. Hassan, M. Ebtekar, Immunological consequence of sulfur mustard exposure, Immunol. Lett. 83 (2002) 151–152.
[6] L. Ghotbi, Z. Hassan, The immunostatus of natural killer cells in people exposed to sulfur mustard, Int. Immunopharmacol. 2 (2002) 981–985.
[7] J.-H. Calvet, P.-H. Jarreau, M. Levame, H. Lorino, A. Harf, I. Macquin-Mavier, Acute and chronic respiratory effects of sulfur mustard intoxication in guinea pig, J. Appl. Physiol. 76 (1994) 681–688.
[8] M.R. Khazdair, M.H. Boskabady, V. Ghorani, Respiratory effects of sulfur mustard exposure, similarities and differences with asthma and COPD, Inhal. Toxicol. 27 (2015) 731–744.
[9] A.T. Koparal, M. Zeytinoğlu, Effects of Carvacrol on a Human Non-Small Cell Lung Cancer (NSCLC) Cell Line, A549, Animal Cell Technology: Basic Appl Aspects, Springer, 2003, pp. 207–211.
[10] M. De Vincenzi, A. Stammati, A. De Vincenzi, M. Silano, Constituents of aromatic plants: carvacrol, Fitoterapia 75 (2004) 801–804.
[11] F. Chen, Z. Shi, K. Neoh, E. Kang, Antioxidant and antibacterial activities of eugenol and carvacrol-grafted chitosan nanoparticles, Biotechnol. Bioeng. 104 (2009) 30–39.
[12] A.G. Guimarães, M.A. Xavier, M.T. de Santana, E.A. Camargo, C.A. Santos, F.A. Brito, et al., Carvacrol attenuates mechanical hypernociception and inflammatory response, Naunyn-Schmiedeberg's Arch Pharmacol. 385 (2012) 253–263.
[13] M.R. Khazdair, V. Ghorani, A. Alavinezhad, M.H. Boskabady, Pharmacological effects of Zataria multiflora Boiss L. and its constituents, focus on their anti-inflammatory, antioxidant and immunomodulatory effects, Fund. Clin. Pharmacol. 32 (2018) 26–50.
[14] A. Jaafari, M. Tilaoui, H.A. Mouse, L.A. M'bark, R. Aboufatima, A. Chait, M. Lepoivre, A. Zyad, Comparative study of the antitumor effect of natural monoterpenes: relationship to cell cycle analysis, Revista Brasileira de Farmacognosia 22 (2012) 534–540.
[15] A. Nostro, T. Papalia, Antimicrobial activity of carvacrol: current progress and future prospectives, Recent Patents Anti-Infect. Drug Discov. 7 (2012) 28–35.
[16] P. Landa, L. Kokoska, M. Pribylova, T. Vanek, P. Marsik, In vitro anti-inflammatory activity of carvacrol: inhibitory effect on COX-2 catalyzed prostaglandin E 2 biosynthesisb, Arch. Pharm. Res. 32 (2009) 75–78.
[17] B. Aristatile, A.H. Al-Assaf, K.V. Pugalendi, Carvacrol suppresses the expression of inflammatory marker genes in D-galactosamine-hepatotoxic rats, Asia. Pacific J. Trop. Med. 6 (2013) 205–211.
[18] M.d.S. Lima, L.J. Quintans-Júnior, W.A. de Santana, C. Martins Kaneto, M.B. Pereira Soares, C.F. Villarreal, Anti-inflammatory effects of carvacrol: evidence for a key role of interleukin-10, Eur. J. Pharmacol. 699 (2013) 112–117.
[19] M. Kara, S. Uslu, F. Demirci, H.E. Temel, C. Baydemir, Supplemental carvacrol can reduce the severity of inflammation by influencing the production of mediators of inflammation, Inflammation 38 (2015) 1020–1027.
[20] S. Jalali, M.H. Boskabady, A.H. Rohani, A. Eidi, The effect of carvacrol on serum cytokines and endothelin levels of ovalbumin sensitized guinea-pigs, Iran. J. Basic Med. Sci. 16 (2013) 615.
[21] M.H. Boskabady, A. Tabatabaee, S. Jalali, Potential effect of the extract of Zataria multiflora and its constituent, carvacrol, on lung pathology, total and differential WBC, IgE and eosinophil peroxidase levels in sensitized guinea pigs, J. Func. Foods. 11 (2014) 49–61.
[22] L.G. Mahtaj, A. Feizpour, M. Kianmehr, M. Soukhtanloo, M.H. Boskabady, The effect of carvacrol on systemic inflammation in guinea pigs model of COPD induced by cigarette smoke exposure, Pharmacol. Rep. 67 (2015) 140–145.
[23] S. Bajaj, D. Singla, N. Sakhuja, Stability testing of pharmaceutical products, J. Appl. Pharm. Sci. 2 (2012) 129–138.
[24] R.M. Molloy, R.I. Mc Connell, J.V. Lamont, S.P. FitzGerald, Automation of biochip array technology for quality results, Clin. Chem. Lab. Med. 43 (2005) 1303–1313.
[25] N.C. Mishra, G.R. Grotendorst, R.J. Langley, S.P. Singh, S. Gundavarapu, W.M. Weber, J.C. Pena-Philippides, M.R. Duncan, M.L. Sopori, Inhalation of sulfur mustard causes long-term T cell-dependent inflammation: possible role of Th17 cells in chronic lung pathology, Int. immunopharmacol. 13 (2012) 101–108.
[26] D. Attaran, S.M. Lari, M. Towhidi, H.G. Marallu, H. Ayatollahi, M. Khajehdaluee, M. Ghanei, R. Basiri, Interleukin-6 and airflow limitation in chemical warfare patients with chronic obstructive pulmonary disease, Int. J. Chron. Obstructive Pulmon. Dis. 5 (2010) 335.
[27] F. Cowan, W. Smith, T. Moran, M. Paris, A. Williams, A. Sciuto, Sulfur mustard-and phosgene-increased IL-8 in human small airway cell cultures, Army Med. Res. Inst. Chem. Def. Aberdeen Proving Ground Md. (2004).
[28] K. Ahmadi, G. Solgue, Cytokine pattern in sera and broncho-alveolar lavage six months after single exposure to sulfur mustard, Med. J. Islam. Repub. Iran (MJIRI) 20 (2006) 52–56.
[29] S. Pourfarzam, T. Ghazanfari, R. Yaraee, H. Ghasemi, Z.M. Hassan, S. Faghihzadeh, S.K. Ardestani, A. Kariminia, F. Fallahi, M.R. Soroush, Serum levels of IL-8 and IL-6 in the long term pulmonary complications induced by sulfur mustard: Sardasht-Iran Cohort Study, Int. immunopharmacol. 9 (2009) 1482–1488.
[30] R. Yaraee, T. Ghazanfari, M. Ebtekar, S.K. Ardestani, A. Rezaei, A. Kariminia, S. Faghihzadeh, A. Mostafaie, M.R. Vaez-Mahdavi, M. Mahmoudi, Alterations in serum levels of inflammatory cytokines (TNF, IL-1alpha, IL-1beta and IL-1Ra) 20years after sulfur mustard exposure: Sardasht-Iran cohort study, Int. immunopharmacol. 9 (2009) 1466–1470.
[31] M. Ezhumalai, N. Ashokkumar, K.V. Pugalendi, Combination of carvacrol and rosiglitazone ameliorates high fat diet induced changes in lipids and inflammatory markers in C57BL/6J mice, Biochimie 110 (2015) 129–136.
[32] M. da Silva Lima, L.J. Quintans-Júnior, W.A. de Santana, C.M. Kaneto, M.B.P. Soares, C.F. Villarreal, Anti-inflammatory effects of carvacrol: evidence for a key role of interleukin-10, Eur. J. Pharmacol. 699 (2013) 112–117.
[33] M. Kianmehr, A. Rezaei, M.H. Boskabady, Effect of carvacrol on various cytokines genes expression in splenocytes of asthmatic mice, Iran. J. Basic Med. Sci. 19 (2016) 402.
[34] M.H. Boskabady, J. Farhadi, The possible prophylactic effect of Nigella sativa seed aqueous extract on respiratory symptoms and pulmonary function tests on chemical war victims: a randomized, double-blind, placebo-controlled trial, J. Alternat. Complemen. Med. 14 (2008) 1137–1144.
[35] A. Emad, G.R. Rezaian, The diversity of the effects of sulfur mustard gas inhalation on respiratory system 10 years after a single, heavy exposure analysis of 197 cases, Chest J. 112 (1997) 734–738.
[36] K. Hoseini, S. Alavi, A. Abedi, Reversibility of airflow obstruction in chronic obstructive disease secondary to sulfur mustard gas injury, 1999.
[37] A. Alavinezhad, M.R. Khazdair, M.H. Boskabady, Possible therapeutic effect of carvacrol on asthmatic patients: a randomized, double blind, placebo-controlled, Phase II clinical trial, Phytother. Res. 32 (2017) 151–159.
[38] M. Boskabady, P. Jandaghi, Relaxant effects of carvacrol on guinea pig tracheal chains and its possible mechanisms, Die Pharmazie-An Int. J. Pharm. Sci. 58 (2003) 661–663.
[39] Y.M. Silva, M.T. Silva, P.A. Sampaio, J.S. Quintans, L.J. Quintans Junior, L.A. Ribeiro, Relaxant effect of carvacrol, citronellal and p-cymene, monoterpenes present in Thymus and Cymbopogon species, in guinea-pig trachea: a comparative study, J. Med. Plants Res. 8 (2014) 881–888.
[40] M. Boskabady, Z. Jafari, I. Pouraboli, The effect of carvacrol on muscarinic receptors of guinea-pig tracheal chains, Phytother. Res. 25 (2011) 530–535.
[41] M.H. Boskabady, H. Tabanfar, Z. Gholamnezhad, H.R. Sadeghnia, Inhibitory effect of Zataria multiflora Boiss and carvacrol on histamine (H1) receptors of guinea-pig tracheal chains, Fund. Clin. Pharmacol. 26 (2012) 609–620.
[42] M.H. Boskabady, M. Kaveh, N. Eftekhar, A. Nemati, Zataria multiflora Boiss and carvacrol affect β2-adrenoceptors of guinea pig trachea, Evid Based Complemen. Alternat. Med. 2011 (2010).