Avicenna Journal of Phytomedicine (AJP)

Short-Communication

Therapeutic effects of *Zataria multiflora* Boiss. extract-loaded chitosan nanoparticles against asthma in rats

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Article history:
Received: Jan 09, 2025
Received in revised form: Jul 20, 2025
Accepted: Jul 22, 2025
Epub ahead of print

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Keywords:
Asthma
Zataria multiflora Boiss
Nanotechnology
Chitosan
Rat

Abstract

Objective: Asthma is one of the most common chronic inflammatory disorders worldwide. *Zataria multiflora* Boiss., widely used in herbal medicine, shows potential efficacy against asthma. In this study, *Z. multiflora* was encapsulated in chitosan nanoparticles to improve its therapeutic effectiveness against asthma in rats.

Materials and Methods: Thirty-six rats were divided to 6 groups including control, asthma (sensitized to ovalbumin (OVA)), and asthmatic groups treated with dexamethasone, empty nano-chitosan, and 8 and 80 mg/kg nano *Z. multiflora* hydroalcoholic extract (Herbarium No: 35314, FUMH) through inhalation. Superoxide dismutase (SOD) and catalase (CAT) activities, malondialdehyde (MDA) concentration, total white blood cells (WBC) counts, interferon-gamma (IFN-γ), interleukin-4 (IL-4) and IFN-γ to IL-4 ratio in bronchoalveolar lavage fluid (BALF) and tracheal responsiveness were assessed.

Results: Nano *Z. multiflora* treatment significantly reduced WBC count and levels of MDA and IL-4 compared to the asthma group. Also, the nano extract of *Z. multiflora* caused a protective effect on elevated tracheal responsiveness to methacholine and ovalbumin.

Conclusion: The therapeutic efficacy of *Z. multiflora* can potentially be enhanced through targeted delivery using chitosan nanoparticles administered via inhalation in asthmatic rats.

Please cite this paper as:
Parvaz N, Mirhosseini M, Saadat S, Amin F, Etemad L. Therapeutic effects of *Zataria multiflora* Boiss. extract-loaded chitosan nanoparticles against asthma in rats. Avicenna J Phytomed, 2025. Epub ahead of print.

Introduction

Asthma is a prevalent inflammatory lung disease affecting individuals of all ages, with higher incidence rates observed in women than men (Kaur and Chupp 2019; Wu et al. 2019). While its exact etiology

remains unclear, both genetic and environmental factors contribute to the disease pathogenesis (Lemanske Jr and Busse 2010). Current treatment strategies focus on symptom management, inflammation control, and pulmonary function improvement, with herbal interventions such as *Zataria multiflora* Boiss. demonstrating significant antiasthmatic potential (Akah et al. 2003; Alavinezhad et al. 2017; Lemanske Jr and Busse 2010; Sagar and Sahoo 2012; Wang et al. 2019).

Zataria multiflora Boiss, a member of the Lamiaceae family indigenous to Iran, Afghanistan, and Pakistan (Kianmehr et al. 2017a), has shown therapeutic efficacy in ameliorating cough and inflammatory pulmonary conditions. Experimental studies indicate that its extract reduces airway inflammation and modulates cytokine profiles in asthma models (Boskabady and Jalali 2013; Boskabady et al. 2013). The plant bioactive components including terpenes, phenols, flavonoids, and particularly carvacrol and thymol are primarily responsible for its pharmacological effects (Boskabady and Gholami Mhtaj 2014; Boskabady et al. 2011).

Nanoparticle-based drug delivery systems enhance therapeutic efficacy by improving drug solubility and bioavailability (Saraf 2010). Their ability to penetrate respiratory mucosa makes them particularly suitable for pulmonary delivery (Yan and Sha 2023). Chitosan nanoparticles offer additional advantages including enhanced drug stability and reduced systemic toxicity (Wang et al. 2011) and have demonstrated potential in augmenting the anti-inflammatory effects of asthma therapeutics like theophylline (Kandasamy et al. 2010; Lee et al. 2006).

Despite established evidence supporting *Z. multiflora* antiasthmatic properties, its formulation with chitosan nanoparticles remains unexplored. This study investigates the potential therapeutic enhancement of *Z. multiflora* through nanoencapsulation.

Materials and Methods

Plant and extract

Zataria multiflora was collected from the mountainous region spanning Yazd and Tabas, Iran. Botanical identification was confirmed by Mr. Joharchi at the Ferdowsi University of Mashhad Herbarium (voucher specimen No. 35314, FUMH). The hydroalcoholic extract was prepared following established phytochemical protocols.

Preparation of *Z. multiflora* extract - loaded chitosan particles

A 1% (w/v) chitosan solution was made in 1% (v/v) acetic acid, and mixed with 1% (v/v) Tween 80 for 2 hr. For drug loading, two concentrations of *Z. multiflora* extract (0.25 and 0.5 g) were prepared in 5 ml of ethanol and added to the chitosan solution, and mixed for 20 min. A 0.5% pentasodium tripolyphosphate solution was added dropwise while stirring for 1 hr, followed by sonication for 10 min. The particles were separated via centrifugation at 14,000 rpm for 20 min, mixed with distilled water, and stored in a freezer at -20°C. Particle loading was measured using a visible-ultraviolet spectrophotometer (PGT 80+, England) in the range of 200 to 800 nm.

Animals and groups

Thirty-six male Wistar rats (200-250 g) were randomly divided into six experimental groups (n=6 per group):

Control group (Ctrl): Received 0.9% sterile saline solution via inhalation.

Asthma group: Sensitized with ovalbumin (1%OVA) via inhalation.

A+CNP group: Treated with empty nano-chitosan post-sensitization via inhalation.

A+ZmCNP8 group: Treated with low concentration (8 mg/kg/day) of *Z. multiflora* extract-loaded chitosan particles via inhalation.

A+ZmCNP80 group: Treated with high concentration (80 mg/kg/day) of *Z. multiflora* extract-loaded chitosan particles via inhalation.

A+DEX group: Treated with 0.03 mg/kg/day dexamethasone via intraperitoneal injection.

Induction of OVA-induced allergic airway inflammation

On days 0 and 8, except for the control group, rats were sensitized with intraperitoneal injections of OVA. Subsequently, from days 14 to 21, the animals were exposed to aerosolized OVA using a nebulizer to induce an airway allergic inflammatory response. This stepwise sensitization and challenge protocol with OVA is a well-established standard model of allergic asthma and has been widely used in previous study (Wang et al. 2012) Following OVA sensitization and challenge, the animals were exposed to chitosan nanoparticles concurrently with the 1% OVA aerosol exposure (Figure 1).

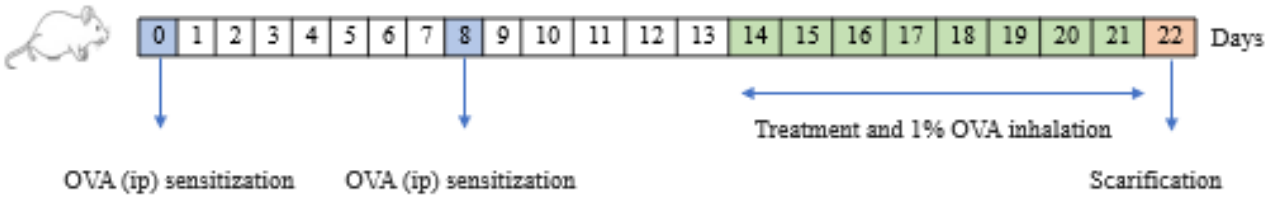

Figure 1. Timetable of OVA exposure and treatments

The aerosol concentration was determined using a calibrated inhalation chamber system. This chamber was equipped with an aerosol generator that produced particles with a mass median aerodynamic diameter (MMAD) of 1–4 μm. The stability of the aerosol concentration during inhalation was monitored in real-time using a RAM-S (Real-Time Aerosol Monitor). Six rats in each group, matched for age and weight (200–250 g), were exposed under identical conditions and received the drug via inhalation. Given their comparable physiological characteristics, their respiratory volumes were presumed to be uniform, ensuring consistent delivery of the drug to all animals.

On day 22, rats were humanely euthanized via intraperitoneal injection of a ketamine-xylazine combination (80 and 10 mg/kg, respectively) (Wang et al. 2012).

Bronchoalveolar lavage fluid (BALF) was obtained by introducing 1 ml saline into the right lung through a cannula, followed by gentle lung massage and aspiration. This procedure was repeated five times (Memarzia et al. 2024).

Measurement of oxidant and antioxidant biomarkers

Antioxidant biomarkers, superoxide dismutase (SOD) and catalase (CAT), and the oxidant biomarker, malondialdehyde (MDA), were measured in BALF which was centrifuged and stored at -80°C prior to analysis (Ghasemi et al. 2023).

Total number of leukocytes and the differential count in BALF

Total white blood cell (WBC) counts were determined using Türk's solution and a Neubauer chamber. Blood smears were prepared for differential cell counting using Wright-Giemsa stain (Shakeri and Boskabady 2017).

Measurement of cytokines levels

The levels of cytokines interleukin 4 (IL-4) and interferon gamma (IFNγ) in the lavage were measured using enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturer's guidelines (Karmania Pars Gen, Kerman, Iran).

Measurement of tracheal responsiveness to methacholine and OVA

Following euthanasia, tracheal segments were isolated and mounted in an organ bath system to measure contractile responses to methacholine hydrochloride ((10−8 to 10−3 M) and OVA (0.1%), with isometric tension recorded at 2-min intervals for methacholine and at 15 min following ovalbumin exposure (Ghorani et al. 2018; Shahabadi et al. 2022).

Statistical analysis

Data were analyzed with GraphPad Prism 8.0. The normality of the data was assessed using the Kolmogorov–Smirnov test. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Results are shown as the mean ± SEM. $p<0.05$ was considered significant.

Results

Successful loading of *Z. multiflora* extract into chitosan particles

The successful loading of *Z. multiflora* extract in chitosan particles was investigated using UV techniques. The maximum absorption of the ethanolic solution of *Z. multiflora* extract was observed at 271 nm, which closely aligns with the findings of Keawchaoon and Yoksan (ʎmax in dimethyl sulfoxide(DMSO) ~ 274 nm) (Keawchaoon and Yoksan 2011). (Figure 2). These results demonstrate the successful loading of *Z. multiflora* extract in chitosan nanoparticles.

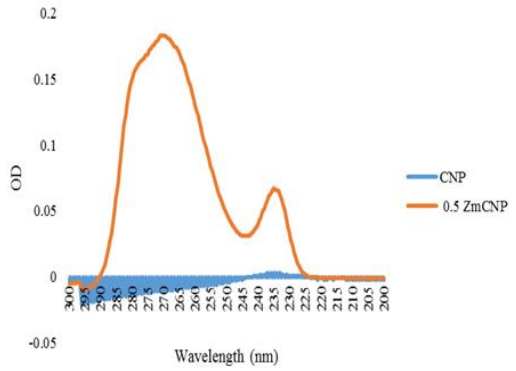

Figure 2. UV spectra of (blue) chitosan nanoparticles (CNP) and (Orange) *Zataria multiflora* extract-loaded chitosan nanoparticles (0.5ZmCNP).

Oxidant and anti-oxidant biomarkers in BALF

The levels of MDA in the Asthma, A+CNP, and A+ZmCNP80 groups were significantly higher than those in the control group ($p<0.01$ to $p<0.001$, Figure 3A). Treatment with a low dose of *Z. multiflora* chitosan nanoparticles (ZmCNP) and dexamethasone (DEX) resulted in decreased MDA levels in the A+ZmCNP8 and A+DEX groups compared to the Asthma group ($p<0.001$, Figure 3A). A significant difference in MDA concentration was observed between the A+CNP group and the groups treated with low and high doses of ZmCNP ($p<0.001$, Figure. 3A). No significant difference in MDA levels was observed between the DEX-treated group and low dose of ZmCNP group; however, a significant difference was found between the DEX- -treated group and the high dose ZmCNP group ($p=0.008$, Figure 3A). There was also a significant difference in MDA levels between low- and high-dose ZmCNP groups ($p=0.02$, Figure. 3A). The SOD activity in the Asthma group was significantly lower than that in the control group ($p<0.05$, Figure 3B), with no significant differences among the treated groups.

CAT activity was significantly reduced in the Asthma, A+CNP, A+ZmCNP8, and A+ZmCNP80 groups compared to the control group ($p<0.01$ and $p<0.001$, Figure 3C). DEX treatment increased CAT activity in the A+DEX group compared to the Asthma group ($p<0.01$, Figure 3C). No significant differences in CAT activity were found among the A+CNP, A+ZmCNP8, and A+ZmCNP80 groups, nor between low- and high-dose ZmCNP groups.

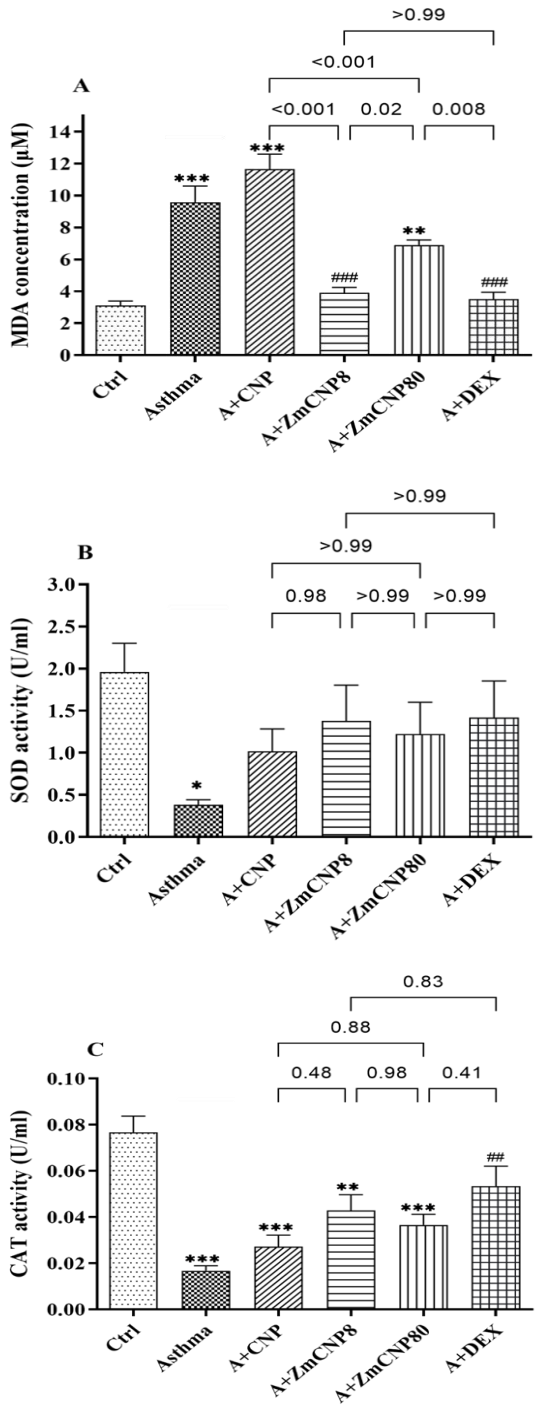

Figure 3. Malondialdehyde (MDA) concentration (A), and superoxide dismutase (SOD) (B) and catalase (CAT) (C) activities in BALF of the control (Ctrl), asthmatic (Asthma), chitosan nanoparticles - treated asthmatic (A+CNP), chitosan nanoparticles of *Zataria multiflora* 8 or 80 mg/kg/day-treated asthmatic (A+ZmCNP8 or A+ZmCNP80), and dexamethasone-treated asthmatic (A+DEX) groups. Data are shown as mean ± SEM (n=6 in each group). ***p<0.001, **p<0.01 and *p<0.05 compared to the Ctrl group, ###p<0.001 and ## p<0.01 compared to the asthma group. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

Total white blood cells in BALF

The total WBC counts were significantly elevated in the Asthma, A+CNP, A+ZmCNP8, and A+ZmCNP80 groups compared to the control group ($p<0.05$ to $p<0.001$). Treatment with ZmCNP or DEX significantly reduced these counts compared to the Asthma group ($p<0.01$ to $p<0.001$). Notably, the A+CNP group showed a significantly higher WBC count than both the low- (A+ZmCNP8, $p<0.001$) and high-dose (A+ZmCNP80, $p=0.01$) ZmCNP treatment groups. Furthermore, while no significant difference was observed between the DEX and A+ZmCNP8 groups, the WBC count in the high-dose ZmCNP group (A+ZmCNP80) remained significantly higher than in the DEX-treated group ($p<0.001$). There was a significant difference in total WBC count between the low- and high-dose ZmCNP groups ($p=0.007$, Figure 4).

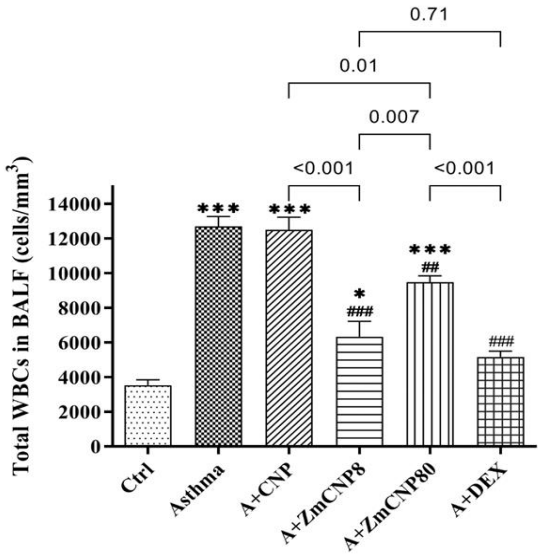

Figure 4. Total white blood cells (WBC) counts in bronchoalveolar lavage fluid (BALF) of the control (Ctrl), asthmatic (Asthma), chitosan nanoparticles - treated asthmatic (A+CNP), chitosan nanoparticles of *Zataria multiflora* 8 or 80 mg/kg/day-treated asthmatic (A+ZmCNP8 or A+ZmCNP80), and dexamethasone-treated asthmatic (A+DEX) groups.. Data are shown as mean ± SEM (n=6 in each group). ***p<0.001 and *p<0.05 compared to the Ctrl group, ###p<0.001 and ##p<0.01 compared to the Asthma group. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

The levels of IL-4 and IFN-γ in BALF

The BALF level of IL-4 in the Asthma and A+CNP groups was significantly higher than that in the control group ($p<0.001$ for both cases). Treatment with ZmCNP and DEX significantly decreased BALF IL-4 levels in the A+ZmCNP8, A+ZmCNP80 and A+DEX groups compared to the Asthma group ($p<0.001$ for all cases). A significant difference in IL-4 levels was observed between the A+CNP group and the groups treated with low and high doses of ZmCNP ($p<0.001$ for both cases). The efficacy of ZmCNP in reducing IL-4 was comparable to that of DEX, as no significant difference was found between these treatment groups Additionally, there was no significant difference in IL-4 levels between the low- and high-dose ZmCNP groups (Figure 5A).

The BALF levels of IFN-γ were significantly lower in the Asthma, A+CNP and A+ZmCNP80 groups compared to the control group ($p<0.05$ to $p<0.001$). Treatment with DEX increased BALF IFN-γ levels in the A+DEX group compared to the Asthma group ($p<0.01$). No significant difference was observed in IFN-γ levels between the ZmCNP-treated groups and the Asthma group, or between the DEX -treated group and the ZmCNP-treated groups. There was also no significant difference in IFN-γ levels between the low- and high-dose ZmCNP groups (Figure 5B).

The IFN-γ to IL-4 ratio was significantly lower in all asthmatic groups compared to the control group ($p<0.01$ to $p<0.001$). Treatment with DEX increased the IFN-γ/IL-4 ratio in the A+DEX group compared to the Asthma group ($p<0.01$). No significant difference was observed in the IFN-γ/IL-4 ratio between the ZmCNP-treated groups and the Asthma group, or between the DEX-treated group and ZmCNP-treated groups. There was also no significant difference in the IFN-γ/IL-4 ratio between low- and high-dose ZmCNP groups (Figure 5C).

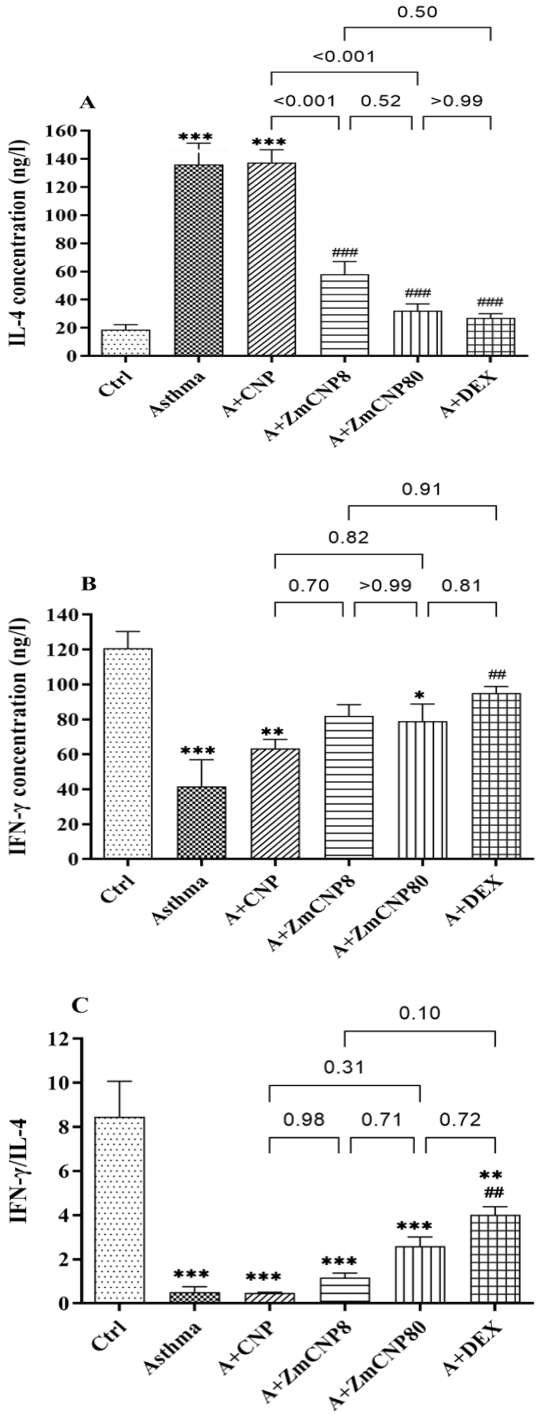

Figure 5. The levels of IL-4 (A) and interferon-gamma (IFN-γ) (B), and IFN-γ to IL-4 ratio (C) in BALF of the control (Ctrl), asthmatic (Asthma), chitosan nanoparticles -treated asthmatic (A+CNP), chitosan nanoparticles of *Zataria multiflora* 8 or 80 mg/kg/day-treated asthmatic (A+ZmCNP8 or A+ZmCNP80), and dexamethasone-treated asthmatic (A+DEX) groups.. Data are shown as mean ± SEM (n=6 in each group). ***p<0.001, **p<0.01 and *p<0.05 compared to the Ctrl group, ###p<0.001 and ##p<0.01 compared to the Asthma group. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

Tracheal responsiveness to methacholine and OVA

OVA-sensitization in the rats of the Asthma group resulted in a significant leftward shift in the methacholine concentration-response curve compared to the control group. Interestingly, treatment with ZmCNP or DEX in the asthmatic rats shifted the methacholine concentration-response curves markedly to the right compared to the Asthma group (Figure 6).

Tracheal responsiveness to OVA in the Asthma and A+CNP groups was significantly higher than that in the control group ($p<0.001$ for both cases). Moreover, a significant difference was found between the Asthma group and the groups treated with low ($p<0.001$) and high ($p<0.01$) doses of ZmCNP or with DEX ($p<0.001$) in terms of tracheal responsiveness to OVA (Figure 7). A significant difference was found between the A+CNP group and the groups treated with a low dose of ZmCNP ($p=0.004$.). However, no significant difference was observed between the A+CNP group and the groups treated with a high dose of ZmCNP ($p=0.05$). No significant difference was observed between the group treated with DEX and the groups treated with low or high doses of ZmCNP. There was no significant difference in tracheal responsiveness to OVA between the low- and high-dose ZmCNP groups (Figure 7).

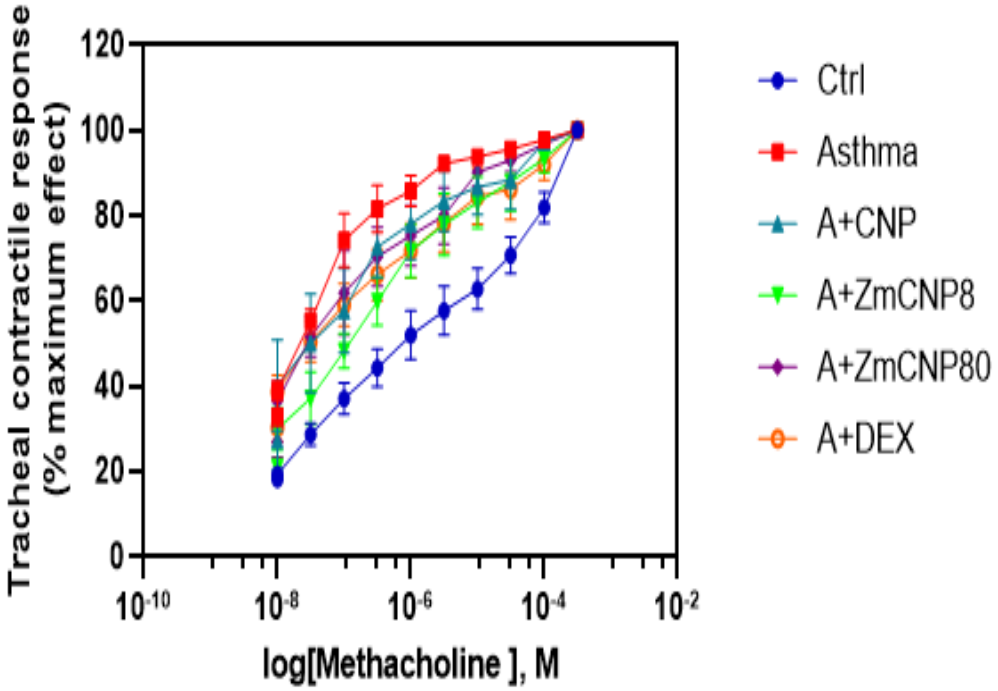

Figure 6. Cumulative log concentration-response curves of methacholine-induced contraction of isolated trachea in the control (Ctrl), asthmatic (Asthma), chitosan nanoparticles -treated asthmatic (A+CNP), chitosan nanoparticles of Zataria multiflora 8 or 80 mg/kg/day-treated asthmatic (A+ZmCNP8 or A+ZmCNP80), and dexamethasone-treated asthmatic (A+DEX) groups. Data are shown as mean ± SEM (n=5 in each group).

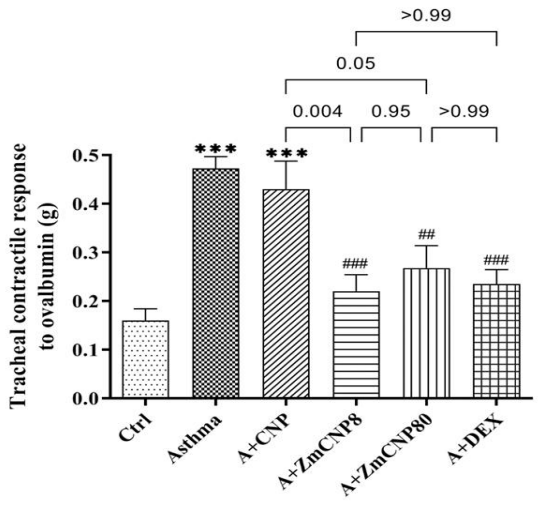

Figure 7. The response to OVA in the control (Ctrl), asthmatic (Asthma), chitosan nanoparticles -treated asthmatic (A+CNP), chitosan nanoparticles *of Zataria multiflora* 8 or 80 mg/kg/day-treated asthmatic (A+ZmCNP8 or A+ZmCNP80), and dexamethasone-treated asthmatic (A+DEX) groups. Data are shown as mean ± SEM (n=8 in each group). ***p<0.001 compared to the Ctrl group, ###p<0.001 and ##p<0.01 compared to the asthma group. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

Discussion

This study investigated the effects of *Z. multiflora* Boiss-loaded chitosan nanoparticles on biomarkers in BALF and tracheal responsiveness in OVA-induced asthmatic rats. Key findings included elevated levels of MDA, WBC, and IL-4 alongside elevated activities of SOD and CAT, and lower level of IFN-γ compared to controls. These changes reflect heightened oxidative stress and inflammation in asthma. Previous studies support these observations, demonstrating increased MDA level in both asthmatic animal models and human patients, which underscores the role of oxidative stress in asthma pathophysiology (Ahmad et al. 2012; Bai et al. 2019; Boskabady et al. 2021; Shakeri et al. 2017). The results suggest that *Z. multiflora* may exert anti-inflammatory and antioxidant effects, potentially offering therapeutic benefits for asthma management.

SOD and CAT are crucial antioxidant enzymes in pulmonary systems that regulate reactive oxygen species (ROS) homeostasis (Li et al. 2014). Research demonstrates significantly reduced SOD activity in both BALF and serum of OVA-sensitized rats, contributing to bronchial epithelial damage through apoptotic pathways and consequent airway dysfunction (Bai et al. 2019; Boskabady et al. 2021; Comhair et al. 2005; Shakeri et al. 2017). Notably, clinical observations reveal variable SOD activity patterns in asthmatic patients - including decreased (Comhair et al. 2000), elevated (Kurosawa et al. 1993), and unaltered levels (Powell et al. 1994) underscoring its multifaceted role in asthma pathogenesis.

Similarly, diminished CAT activity has been associated with exacerbated oxidative stress and persistent airway inflammation in asthma pathogenesis (Ghosh et al. 2006). Multiple studies document reduced CAT levels across biological matrices, including serum and BALF (Bai et al. 2019; Boskabady et al. 2021; Shakeri et al. 2017), as well as in lung tissue specimens from asthmatic animal models (Alrumaihi et al. 2020; Dalouchi et al. 2021).

Elevated total WBC counts have been consistently observed in both ovalbumin-exposed (Boskabady and Jalali 2020; Shakeri et al. 2017) and paraquat-exposed animal models (Amin et al. 2025; Amin et al. 2021), demonstrating the inflammatory effects induced by these agents.

OVA-sensitized guinea pigs demonstrate elevated IL-4 and reduced IFN-γ levels, indicating a Th2-polarized immune response (Boskabady et al. 2013). Chronic airway inflammation in asthma is characterized by increased Th2 lymphocytes and their associated cytokines (IL-4, IL-5, IL-9, and IL-13), coupled with decreased Th1 lymphocytes and cytokines (IL-2, IFN-γ, and IL-12) (Kianmehr et al. 2017a). This cytokine imbalance highlights asthma's inflammatory pathogenesis and suggests the therapeutic potential of

antioxidant enzymes (SOD and CAT) in mitigating associated oxidative stress.

Treatment with low-dose ZmCNP significantly decreased MDA concentrations versus the asthma group consistent with prior studies demonstrating *Z. multiflora* extract efficacy in reducing oxidative stress in COPD guinea pig models and paraquat-exposed rats (Boskabady and Gholami Mhtaj 2014; Heydari et al. 2021). This enhanced therapeutic effect likely stems from the nanoparticle formulation's reduced particle size, which improves pulmonary absorption efficiency.

No significant alterations in SOD activity were detected following treatment, aligning with clinical observations of stable SOD levels in asthmatic patients receiving *Z. multiflora* therapy (Alavinezhad et al. 2020). In contrast, paraquat-exposed animals demonstrated increased SOD activity after administration of higher *Z. multiflora* doses (Amin et al. 2021).

Only dexamethasone treatment significantly enhanced CAT activity relative to the asthma group, implying that nano-*Zataria multiflora* may lack efficacy in modulating this antioxidant enzyme. This contrasts with prior findings demonstrating CAT activity elevation in paraquat-exposed rats treated with higher *Z. multiflora* doses (Amin et al. 2021; Heydari et al. 2021), potentially explaining the observed divergence from current results.

Zataria multiflora extract has demonstrated efficacy in normalizing total WBC counts across multiple experimental models including asthma (Alavinezhad et al. 2020), COPD (Boskabady et al. 2014b), and paraquat exposure (Amin et al. 2021). Notably, emerging evidence suggests that nano-formulated herbal medicines may achieve enhanced therapeutic effects at reduced doses compared to their crude extracts, potentially due to improved bioavailability and targeted delivery (Park et al. 2020). This dose-sparing effect supports the pharmacological advantage of nanoparticle-based herbal formulations.

Dexamethasone and both doses of nano-*Zataria multiflora* significantly reduced IL-4 concentrations, but only dexamethasone increased IFN-γ levels and IFN-γ to IL-4 ratio. Gene expression analysis in splenocytes from treated asthma groups indicated a significant decrease in IL-4 and an increase in IFN-γ and IFN-γ to IL-4 ratio compared to untreated groups (Kianmehr et al. 2017a). Previous studies have corroborated these findings, showing that *Z. multiflora* extract elevates IFN-γ and IFN-γ to IL-4 ratio and decreases IL-4 levels in various models of asthma (Boskabady et al. 2013; Kianmehr et al. 2017b). The therapeutic effects of *Z. multiflora* are enhanced when encapsulated in nanoparticles, as demonstrated by improved bioavailability and stability, leading to better accumulation in the lungs. This study aligns with other research indicating that nano-formulations of herbal medicines can be more effective at lower doses than their unformulated counterparts. For instance, treatments with curcumin-loaded nanoparticles and other herbal extracts have shown significant reductions in Th2 cytokines, such as IL-4 and IL-5, while increasing IFN-γ levels. (Wang et al. 2012). The levels of IL-4, IL-5, IL-13 and IL-25 were diminished in allergic asthmatic animals treated by glycyrrhizic acid-Poly D,L-lactide-co-glycolic acid(PLGA) nanoparticles (Chen et al. 2022). In the asthmatic mouse treated by andrographolide nanoparticles, the level of IL-4 and IL-5 was more decreased than free andrographolide treatment group. Also, this study showed that the route of administration can be an essential factor in drug delivery, and administration of andrographolide nanoparticles by pulmonary rout was more effective compared to oral route (Chakraborty et al. 2019). The results of asthmatic mice treated with two doses of isoliquiritigenin self-nanoemulsion (5 and 10 mg/kg) showed a significant reduction in IL-4 and IL-5 levels as well as elevated BALF level of IFN-γ compared to free isoliquiritigenin (Cao et

al. 2020). The potential of chrysin loaded PLGA-nanoparticle in reduction of Th2-cytokines was higher than free chrysin in allergic asthmatic BALB/C mouse (Roy et al. 2020). Treatment of asthmatic mice with ferulic acid loaded chitosan nanoparticle via inhalation reduced IL-5, tumor necrosis factor alpha (TNF-α), and IL-13 levels compared to ferulic acid which could be linked to the small size of nanoparticles (Dhayanandamoorthy et al. 2020). The mRNA levels of Th2-cytokines levels (IL-4, IL-5 and IL-13) were reduced in asthmatic animal model treated by bavachinin-loaded PEG-PLGA nanoparticles (Wang et al. 2018). Baicalein encapsulated/loaded chitosan-nanoparticle increased IL-12 and decreased IL-5 and controlled immune-allergy-inflammatory responses in mouse model of allergic asthma (Wang et al. 2021). The results of mentioned studies and this research altogether denote that encapsulation of herbal medicines in nano particles can enhance their anti-inflammatory activity in asthma.

Additionally, this study found that both dexamethasone and nano-*Zataria multiflora* provided protective effects against elevated tracheal responsiveness to methacholine and OVA. These results confirm earlier findings regarding the relaxant effects of *Z. multiflora* on tracheal smooth muscle, attributed to its anti-inflammatory properties (Boskabady and Jalali 2013; Boskabady et al. 2014a). Overall, the encapsulation of *Z. multiflora* in chitosan nanoparticles enhances its therapeutic efficacy in asthma management by improving drug delivery and effectiveness at lower doses.

Chitosan nanoparticles have emerged as a promising method for enhancing herbal drug delivery, particularly in the treatment of asthma. Their small size, low toxicity, biocompatibility, and biodegradability allow for effective accumulation in the lungs, improving the therapeutic effects of herbal medicines like *Z. multiflora*.

Recent studies indicate that chitosan nanoparticles can regulate key inflammatory and anti-inflammatory markers in asthma management, demonstrating a preventive effect on various biological parameters such as white blood cell count and cytokine levels in sensitized rats. The inhalation of chitosan nanoparticle-loaded herbal extracts has successfully addressed challenges related to drug elimination from the lungs. The findings suggest that these nanoparticles can significantly improve the efficacy of herbal treatments for asthma by enhancing their absorption and therapeutic outcomes.

As research progresses, the potential for using nano-sized herbal medicines in asthma treatment appears promising, paving the way for innovative therapeutic strategies in managing this chronic condition.

Acknowledgment

The authors gratefully acknowledge Rafsanjan University of Medical Sciences and Mashhad University of Medical Sciences for their valuable support in conducting this research.

Conflicts of interest

The authors declare that they have no competing interests.

Funding

No Funding.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

The animal studies in this project were authorized by the Animal Ethics Committee of Rafsanjan University of Medical Sciences and carried out in accordance with the Guidelines for the Care of Laboratory Animals in Research (IR.RUMS.REC.1398.171).

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