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Myrtenol Ameliorates Recognition Memories' Impairment and Anxiety-Like Behaviors Induced by Asthma by Mitigating Hippocampal Inflammation and Oxidative Stress in Rats

Mohammad Abbas Bejeshk[a, b, c] Amir Hashem Aminizadeh[c] Elham Jafari[d] Sina Motamedi[a] Iman Zangiabadi[e] Ahmad Ghasemi[f] Mazyar Fathi[a] Akram Nezhadi[g] Faezeh Akhgarandouz[a, h] Fatemeh Bejeshk[a, i] Leila Mohammadi[j] Fatemeh Mohammadi[k] Mohammad Amin Rajizadeh[a, b, c]

[a]Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran; [b]Physiology Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran; [c]Department of Physiology and Pharmacology, Afzalipour Medical Faculty, Kerman University of Medical Sciences, Kerman, Iran; [d]Department of Pathology and Pathology and Stem Cells Research Center, Kerman University of Medical Sciences, Kerman, Iran; [e]Department of Anatomy, Afzalipour Medical Faculty, Kerman University of Medical Sciences, Kerman, Iran; [f]Department of Physiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran; [g]Cognitive and Neuroscience Research Center, Aja University of Medical Sciences, Tehran, Iran; [h]Department of Biology, Faculty of Sciences, Shahid Bahonar University of Kerman, Kerman, Iran; [i]Department of Educational Sciences and Psychology, Kerman Branch, Islamic Azad University, Kerman, Iran; [j]Department of Computer Engineering, Yazd University, Yazd, Iran; [k]Sirjan School of Medical Sciences, Sirjan, Iran

Keywords
Asthma · Myrtenol · Cognitive impairment · Inflammation · Oxidative stress

Abstract

Introduction: Asthma is related to neurochemical alterations which affect brain functions and lead to anxiety and cognitive dysfunctions. Myrtenol has sparked considerable interest due to its pharmacological effects, especially for the remediation of chronic disorders. Thus, the present research was designed to evaluate the impacts of myrtenol on anxiety-like behaviors, cognitive declines, inflammation, and oxidative stress in the hippocampus of asthmatic rats. ***Methods:*** Rats were allocated to five groups: control, asthma, asthma/vehicle, asthma/myrtenol, and asthma/budesonide. Asthma was elicited in the rats by ovalbumin, and the animals were then exposed to myrtenol inhalation. Anxiety-like behavior and memory were assessed by elevated plus maze (EPM) and novel object and location recognition tests. Interleukins (interleukin-6, -17, and -10), tumor necrosis factor α

Fatemeh Mohammadi and Mohammad Amin Rajizadeh contributed equally to this work.

Karger@karger.com
www.karger.com/nim

Correspondence to:
Mohammad Amin Rajizadeh, aminrajizadeh@yahoo.com

Karger

(TNF-α), and oxidative stress biomarkers such as malondialdehyde (MDA), superoxide dismutase (SOD), Glutathione peroxidase (GPX), and total antioxidant capacity (TAC) in the hippocampus were assessed by the ELISA method. ***Results:*** The levels of IL-6, IL-17, TNF-α, and MDA decreased, but GPX, SOD, and TAC levels increased in the hippocampus of asthmatic animals due to myrtenol inhalation. ***Conclusion:*** Myrtenol diminished asthma-induced anxiety-like behaviors and cognitive deficits in asthmatic rats; these effects might have been typically mediated by a reduction in inflammation and oxidative stress.

Introduction

Asthma is a chronic inflammatory condition in which patients' airways narrow down and swell and may produce extra mucus [1]. Inflammation is an essential feature of the lungs and airways in asthma [2]. Based on recent studies, it affects about 300 million people worldwide, and its prevalence is on the rise, especially in developing countries. It also causes about 250,000 deaths annually [3]. Imbalance in the T-helper type 1/T-helper type 2 cytokines ratio is a characteristic of asthma pathophysiology. T-helper type 2 response leads to the release of IL-5 and IL-17, which is related to inflammatory cell influx and airway hyper-responsiveness [4, 5]. High levels of reactive oxygen species production due to inflammation and oxidative stress in asthma eventually increase the generation of IL-6 [6–9]. Additionally, the production of IL-10 and tumor necrosis factor α (TNF-α) is, respectively, attenuated and enhanced in patients with asthma [10, 11]. It is, therefore, clear that IL-6, IL-10 (as an inflammatory cytokine), and TNF-α play essential roles in the pathogenesis of asthma [7, 11, 12]. In addition, some investigations disclosed the relationship between inflammation, oxidative stress, neurodegenerative and neuropsychiatric diseases such as anxiety and cognitive declines [13, 14]. Thus, the risk of cognitive impairments and, consequently, dementia is raised in asthmatic patients [15, 16]. It has also been shown that chronic asthma results in spatial learning and memory impairment and long-term potentiation in rodents [17, 18]. According to this body of evidence and due to the lack of safe and effective drugs to treat asthma-induced inflammation, oxidative stress, cognitive impairment, finding a new therapeutic strategy with fewer side effects for treating neurological dysfunctions in asthmatic patients seems necessary. The beneficial effects of herbal medicine have been known for years. Herbal medicines possess several therapeutic properties such as antioxidant, anti-inflammatory, anticancer, antimicrobial, and immunomodulatory effects [19–21]. Herbal medicines utilized to cure respiratory diseases such as pulmonary fibrosis have also been investigated [22–24].A plant traditionally used in herbal medicine is myrtle (*Myrtus communis*) [25], with neuroprotective, antioxidant, and anti-inflammatory effects [26, 27]. Leaves, fruits, and oil obtained from the leaves of myrtle have therapeutic effects on lung diseases [28]. Previous studies have shown that the extract obtained from seeds of some plants, including myrtle, decreases lung tissue inflammation and fibrosis [22–24]. Myrtenol is an effective component of myrtle with anxiolytic properties [29]. Myrtenol exerts its antioxidant effects by elevating the activity of superoxide dismutase (SOD) and catalase and preventing lipid peroxidation [30]. It also has anti-inflammatory effects on asthma [31]. Heimfartha et al. [32] showed that myrtenol attenuates cognitive impairment induced by fibromyalgia by maintaining oxidative homeostasis. The antioxidant, anti-inflammatory, and neuroprotective effects of myrtenol have been identified [32, 33]; moreover, we have already shown the beneficial effect of myrtenol on asthma in the previous work of our laboratory [31]. Thus, this study was designed to evaluate the improvement impact of myrtenol inhalation on anxiety, cognitive alterations, inflammation, and oxidative stress in the hippocampus of asthmatic rats.

Materials and Methods

Animals

This study was approved by Ethics Code IR.KMU.REC.1399.603 in Kerman University of Medical Sciences, Kerman, Iran. Thirty-five male Wistar rats (weight range: 200–250 g, 8 weeks old) were kept at the standard temperature (22 ± 2°C), 12-h light/dark cycle, and free access to food and water. These rats were randomly divided into five groups (7 rats in each group).

Animal Grouping, Asthma Induction, and Treatment Protocols

Figure 1 illustrates the experimental protocols. Myrtenol purchased from Sigma Aldrich (Sigma, St. Louis, MO, USA) was dissolved in DMSO (0.5%). On days 0 and 7, ovalbumin (OVA, 1 mg) and aluminum hydroxide (200 μg) dissolved in 0.5 mL of sterile PBS was intraperitoneally injected into the asthmatic rats. The sensitized rats inhaled 1% aerosolized OVA (1 g OVA in 100 mL of sterile PBS in a nebulizer) for 30 min every other day from day 14 to day 42 in a closed chamber (30 × 50 × 60 cm) using a nebulizer [31, 34, 35]. The rat groups were the CTL group (with no interventions); asthma group (received just OVA); asthma/vehicle group

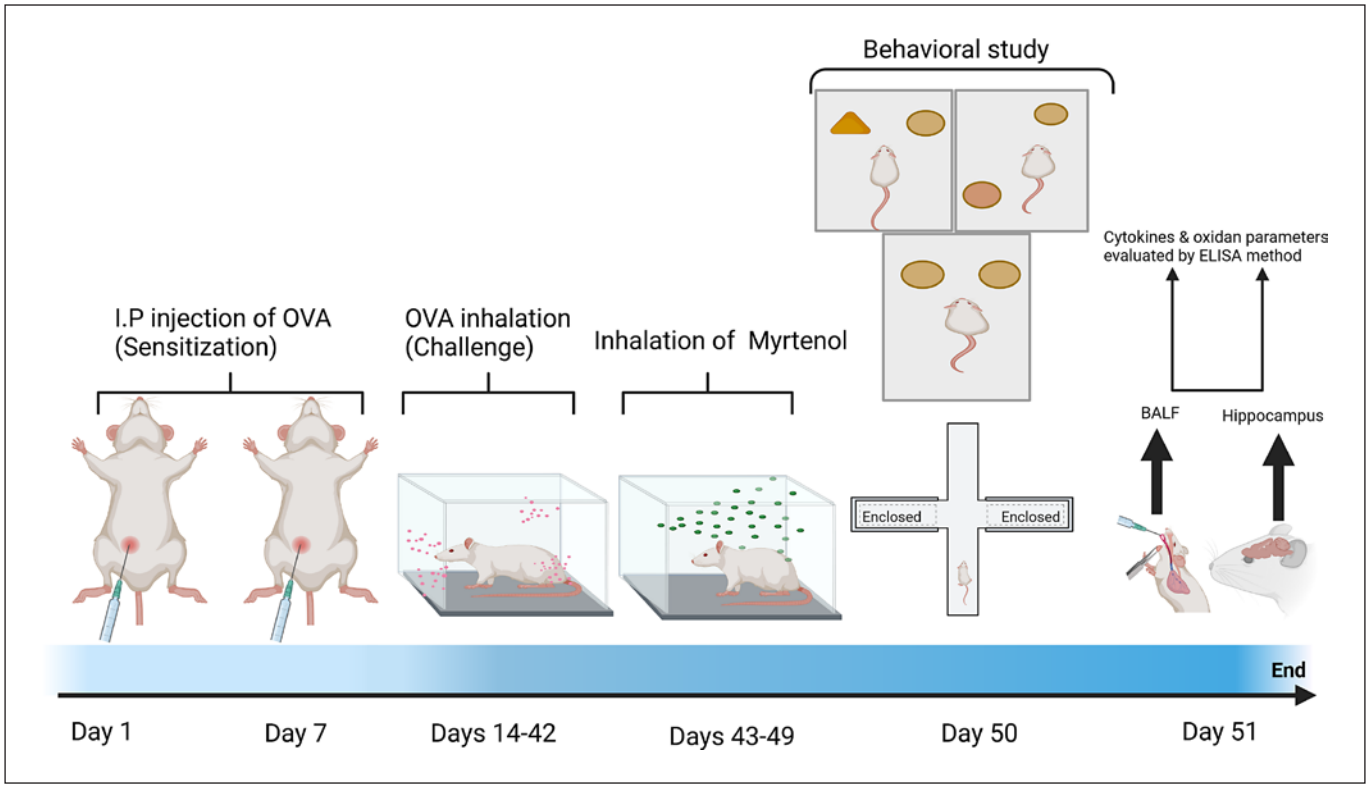

Fig. 1. Time-line diagram showing the protocol used for asthma induction and drug application and behavioral tests in animals. On days 1 and 7, the sensitization phase was performed by intraperitoneal injection of OVA. On days 14–42, the challenge phase was performed by inhalation of OVA. On days 43–49, the animals were treated with myrtenol inhalation. On the 50th day, behavioral tests including EPM (for anxiety evaluation) and novel location and object tests (for recognition memories evaluation) were performed. On the last day, the hippocampus tissue and BALF samples were collected and ELISA method was used to check inflammatory and oxidant factors (created with BioRender.com).

(asthmatic rats that received DMSO); asthma/myrtenol group (asthmatic rats that received myrtenol at a dose of 8 mg/kg); and asthma/budesonide group (asthmatic rats that received budesonide at a dose of 160 μg/kg) as a gold standard [36]. Rats in the treatment groups inhaled myrtenol and budesonide for 30 min per day for seven consecutive days [37]. According to the calculation by the following formula, approximately 2 mg of myrtenol (8 mg/kg) and 40 μg of budesonide (160 μg/kg) reach each animal in 30 min: $D = (C \times MV \times T)/BW$ (D is the estimated dose of the substance [mg/kg body weight], C is the substance concentration in the exposure chamber [mg/L]), T is the duration of exposure [min], BW is the average group body weight [kg], and MV is the animal's minute volume [L/min]) [38].

Elevated plus Maze

The EPM test is a major task for assessing anxiety in rodents. The time spent in the closed arm is an index of high anxiety. The number of head dips (downward movement of rodents' heads toward the floor from the open arms) and the time spent in the open arm are inversely related to anxiety. Two open and two closed arms of this task are made of wood (50 × 50 × 50 cm). The animals' behavior in this task is recorded by a video camera for 5 min [39–41].

Novel Object Recognition Task

In this task, the animals are permitted to detect familiar and novel objects in a box made of wood (60 × 60 × 40 cm) during two phases. The first phase lasts 5 min (training phase), during which the same objects are placed in the same location. The second phase for assessing memory retention is performed 45 min after the training phase and lasts 3 min (test phase). In this phase, the objects are placed in locations similar to the training phase, but one of the previous objects is substituted with a novel object. The time of sniffing or touching the objects with the nose is considered the exploration time and is recorded by a camera. Finally, the time that the rats pay attention to each object divided by the total attention time to both objects multiplied by 100 in the training and test phases is regarded as the discrimination ratio (recognition index) [42–44].

Novel Location Task

In this task, as a hippocampal-dependent task, the rats are permitted to detect the objects in the novel location in a box made of wood (60 × 60 × 40 cm) during two phases. The first phase lasts 5 min (training phase), during which the same objects are placed in the same location. The second phase for assessing memory

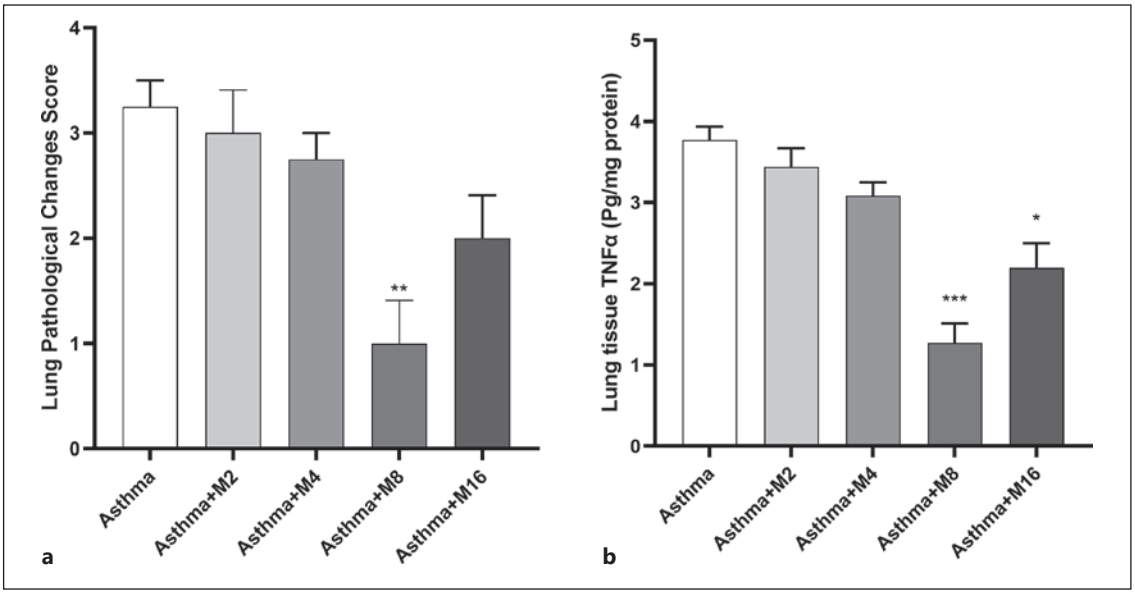

Fig. 2. a, **b** Dose-response study for determining the optimum dose of myrtenol according to the effects on lung pathology and lung tissue TNF-α. The lowest dose that exhibited maximum efficacy was 8 mg/kg among 2, 4, 8, and 16 mg/kg administered. Data are shown as mean ± SEM. $^{**}p < 0.01$ and $^{***}p < 0.001$ versus asthma.

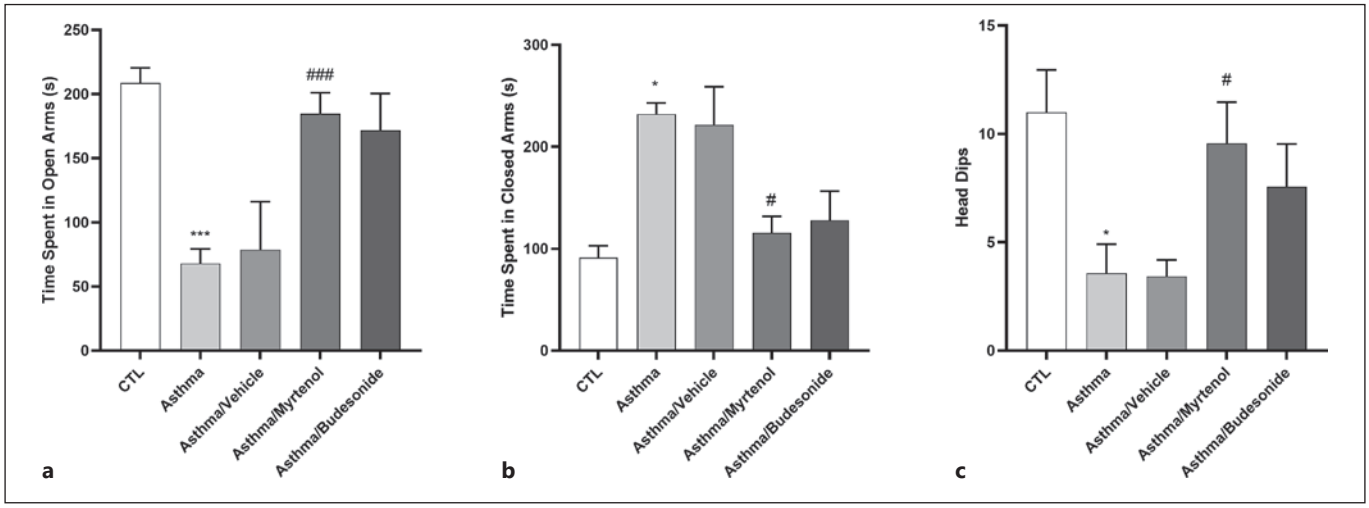

Fig. 3. a–c Effects of myrtenol administration on anxiety-like behaviors in EPM task in asthmatic rats. Asthma decreased time spent in open arm and number of head dips significantly that these changes demonstrated increased anxiety due to asthma. Myrtenol in asthma/myrtenol group significantly reversed the effects of asthma and improved anxiety. One-way ANOVA used for analysis of these data. Data are shown as mean ± SEM. $^{*}p < 0.05$ and $^{***}p < 0.001$ versus CTL; $\#p < 0.05$ and $\#\#\#p < 0.001$ versus asthma.

retention is performed 45 min after the training phase and lasts 3 min (test phase). In this phase, the objects are similar to the training phase but placed in different locations. The time of attention to the objects with the nose and hand is considered as the exploration time and recorded by the camera. Finally, the discrimination ratio (as the recognition index) is calculated similarly to the novel object test [45, 46].

BALF Collection

After scarification, BALF (bronchoalveolar lavage fluid) was collected from the left lungs. Briefly, lungs were lavaged with 2.5 mL of 0.9% sterile saline solution via tracheal tube. The BALF was aspirated slowly after 5 min and centrifuged at 1,500 rpm for 10 min at 4°C. The supernatant was separated and stored at −80°C until analysis for cytokines [47].

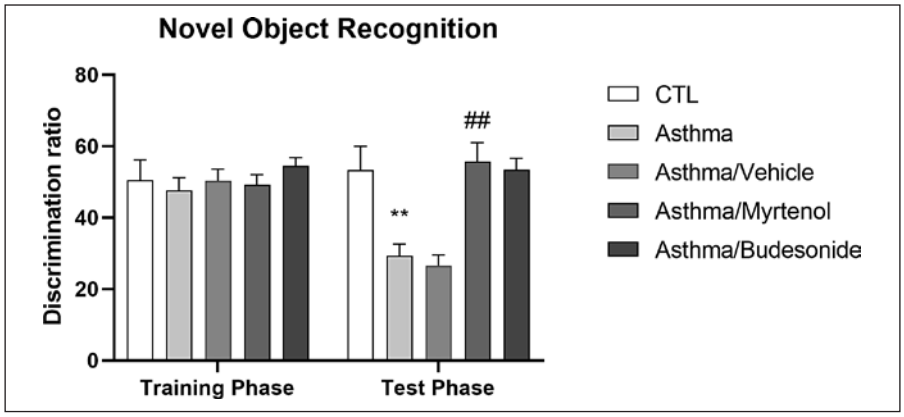

Fig. 4. Effects of asthma and myrtenol administration on novel object recognition test. There was no significant difference in exploration time in training session between all of groups. There was a significant decrease in exploration time in test session in asthma group compared to control group and administration of myrtenol in asthma/myrtenol group increased exploration time compared to asthma group. One-way ANOVA used for statistical analysis. Data are shown as mean ± SEM. $^{**}p < 0.01$ versus CTL; $^{\#\#}p < 0.01$ versus asthma.

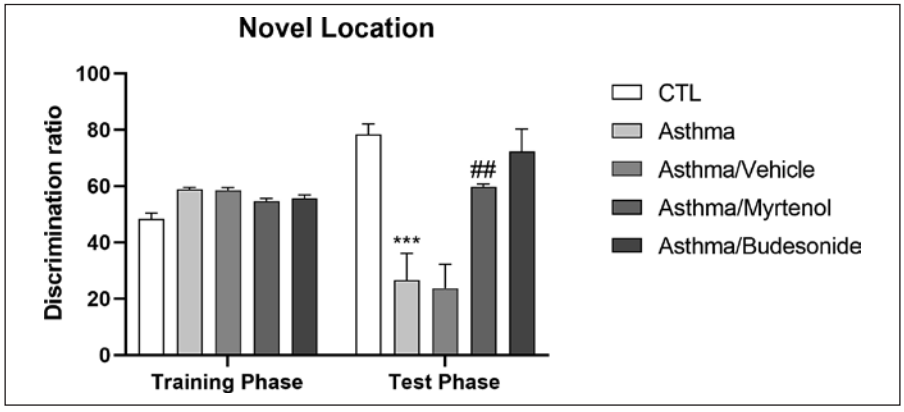

Fig. 5. Effects of asthma and myrtenol on the exploration ratio for the objects during object location task. The exploration ratio is shown for the training and for the test phases. One-way ANOVA and post hoc Tukey's tests used for the analysis of these data. Data were shown as mean ± SEM. $^{***}p < 0.001$ versus CTL group; $^{\#\#}p < 0.01$ versus asthma.

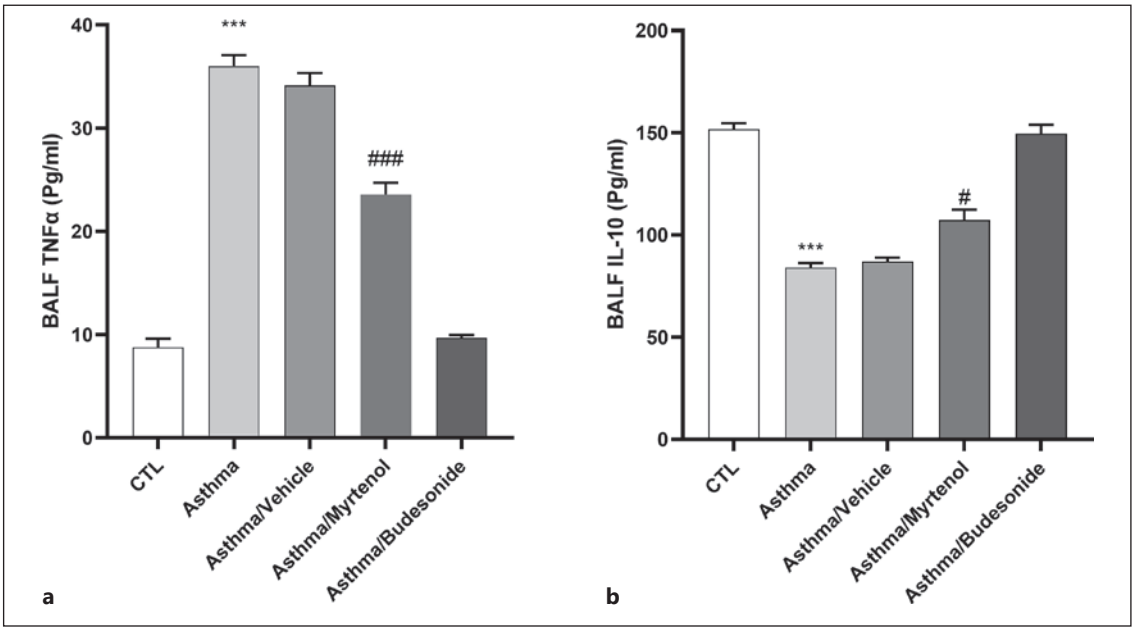

Fig. 6. a, **b** ELISA analyses of inflammatory and anti-inflammatory cytokines in the BALF of different groups. Asthma significantly increased the levels of TNF-α and decreased IL-10 in the BALF. Data are shown as means ± SEM. $^{***}p < 0.001$ versus CTL; $^{\#}p < 0.05$ and $^{\#\#\#}p < 0.001$ versus asthma.

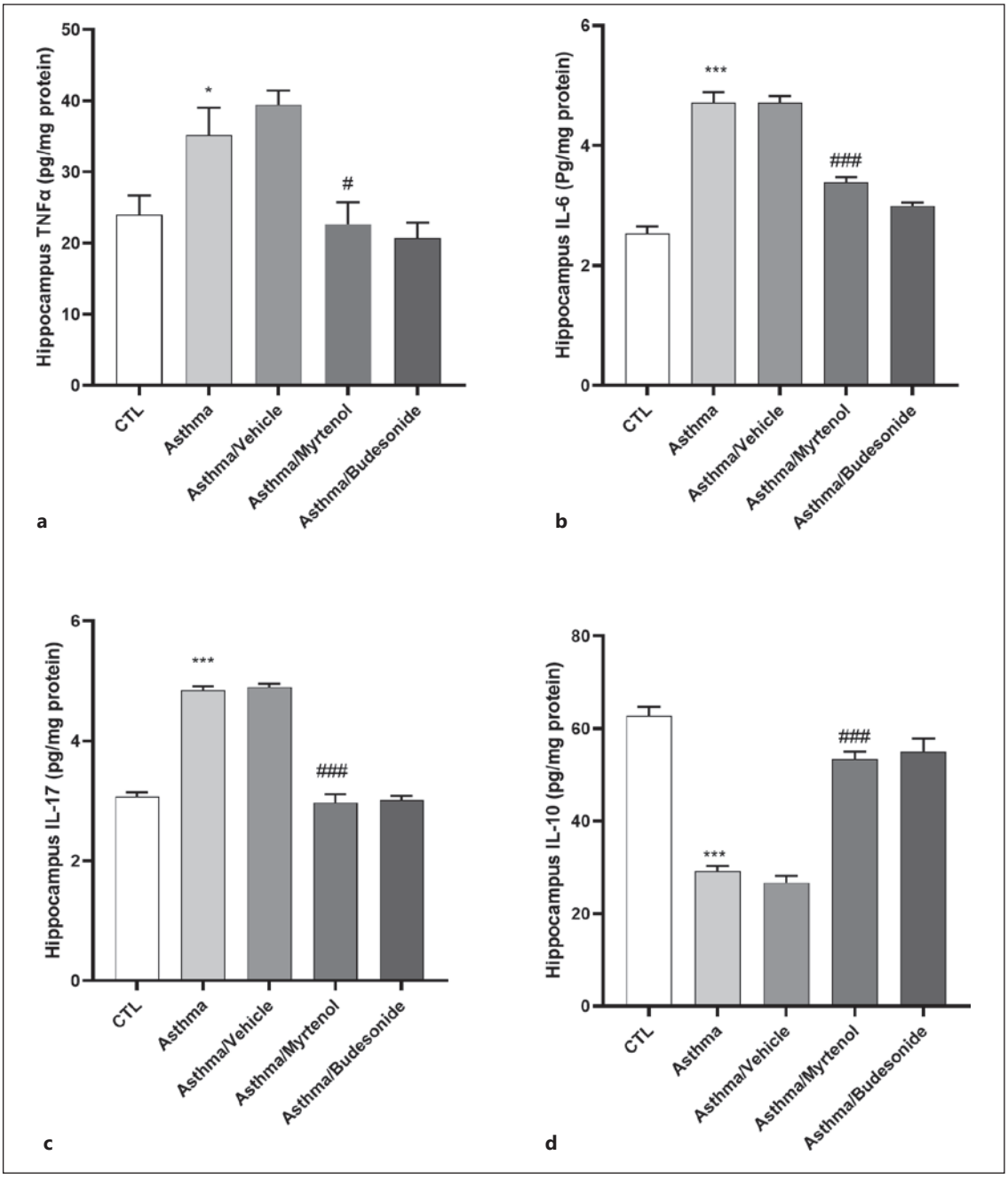

Fig. 7. a–d ELISA analyses of inflammatory and anti-inflammatory cytokines in the hippocampus tissue of different groups. Asthma significantly increased the levels of IL-17, TNF-α, and IL-6 and decreased IL-10 in the hippocampus tissue. Data are shown as means ± SEM. *$p < 0.05$ and ***$p < 0.001$ versus CTL; #$p < 0.05$ and ###$p < 0.001$ versus asthma.

Biochemical Measurements in the Hippocampus

Tissue GPX, SOD, MDA, and TAC were evaluated using their assay kits, based on their respective manufacturers' protocols. The levels of cytokines (TNF-α, IL-6, IL-17, and IL-10) were assessed via ELISA kits and analyzed on an ELISA plate reader (Model ELX-80MS, Biotech, USA).

Lung Histopathology

At the end of the study, the animals were sacrificed under deep anesthesia by administration of ketamine (80 mg/kg) and xylazine (50 mg/kg), and their right lungs were harvested and immersed in 10% formalin. Then, slices were sectioned and stained with hematoxylin/eosin. The sections were examined by a pathologist. The

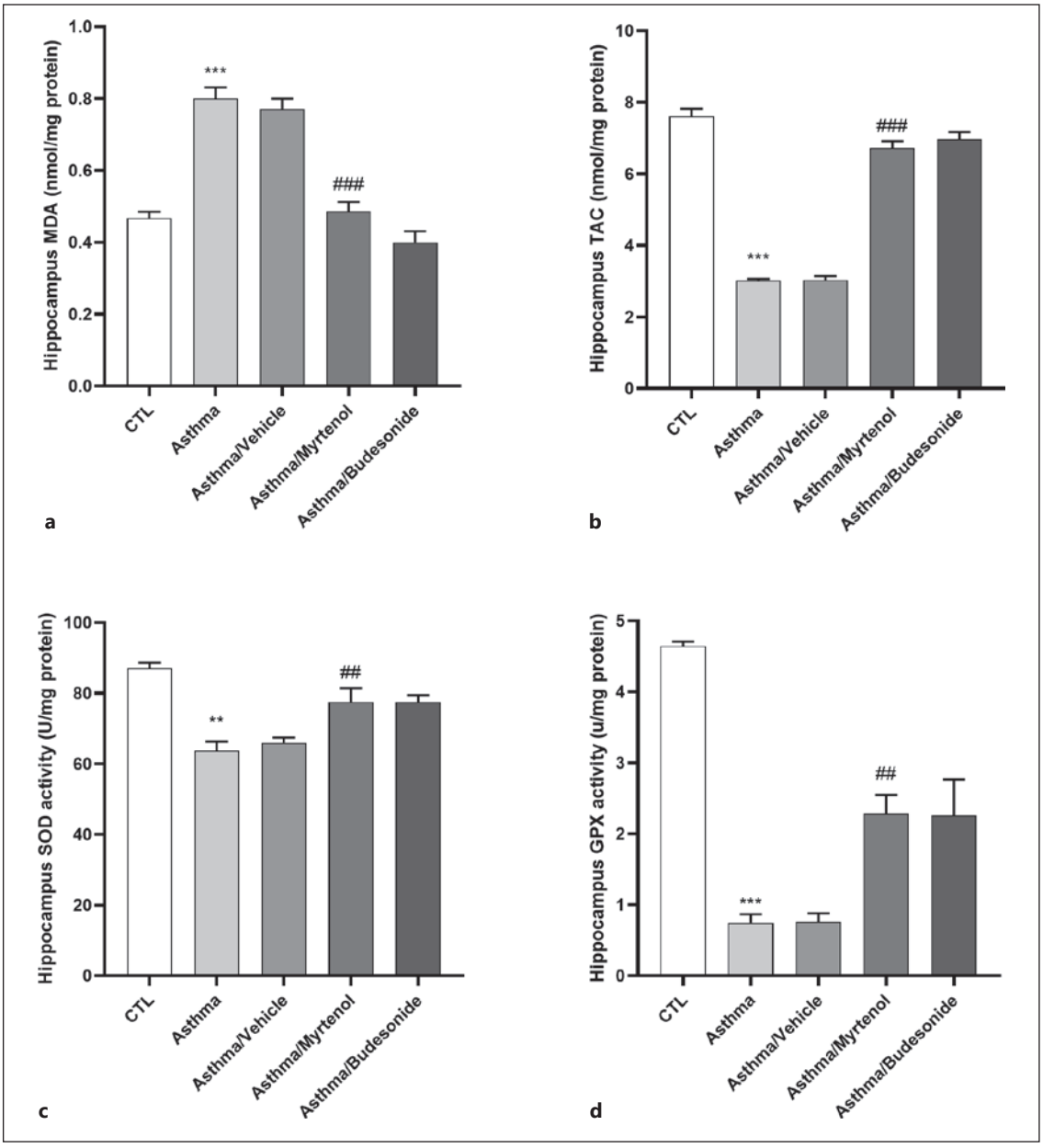

Fig. 8. a–d Alterations of the MDA, TAC, SOD, and GPX in the hippocampus tissue in different groups of the study. Data are shown as means ± SEM. $^{**}p < 0.01$ and $^{***}p < 0.001$ versus CTL; $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ versus asthma.

pathological indices evaluated in the lungs included the following: 0 = no lesion, 1 = slight, 2 = mild, 3 = moderate, and 4 = severe lesions [31].

Dose-Response Study

For the dose-response study, myrtenol at doses of 2, 4, 8, and 16 mg/kg was administered by inhalation to an asthmatic group for a week. The lowest dose that revealed maximum efficacy in diminishing inflammation in biochemistry (tissue TNF-α levels) and histopathology (interstitial inflammation) was chosen as the optimum dose (8 mg/kg) (Fig. 2). This dose was then used for the rest of the study.

Statistical Analysis

The data are reported as mean ± SEM. The Shapiro-Wilk test was performed to check the normality of the data. A one-way analysis of variance followed by Tukey's post hoc test was used for data analysis. The significance level was set to $p < 0.05$ [48].

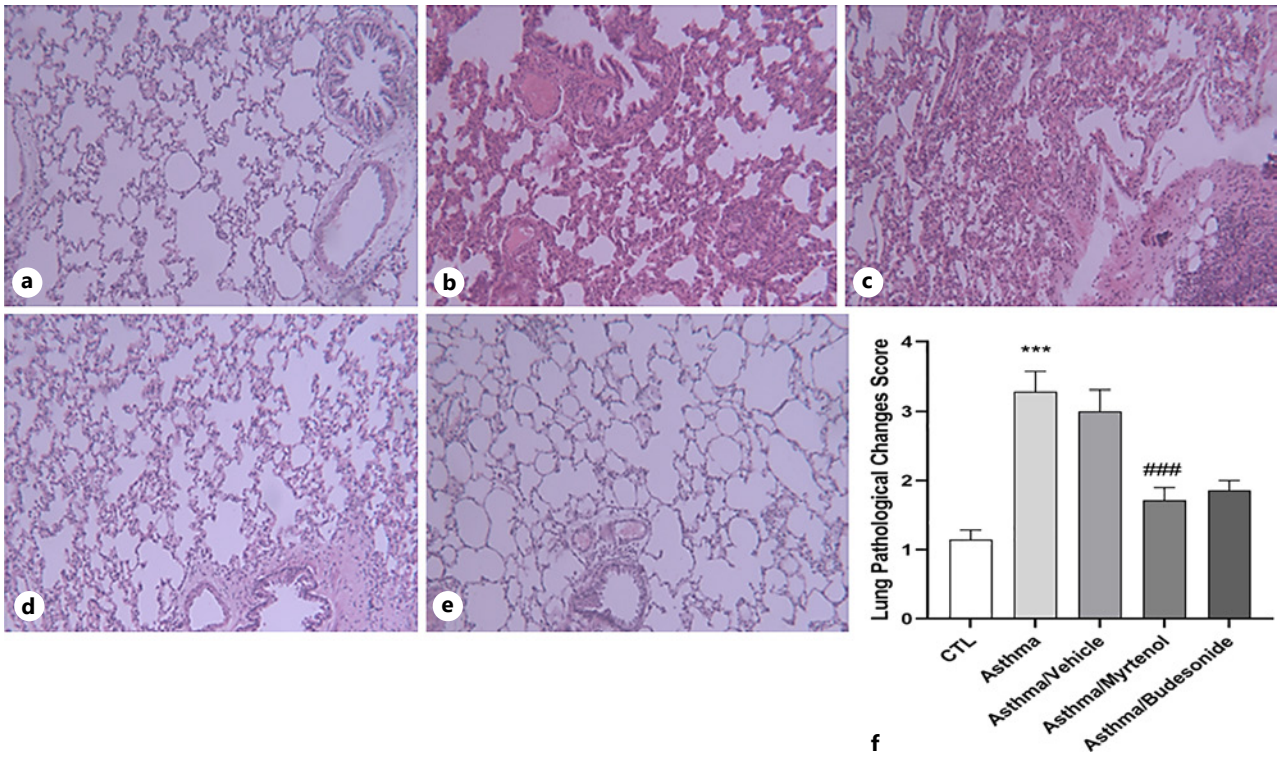

Fig. 9. Micrographs of the lung stained with H&E showing pathologic changes in the bronchial epithelium including inflammation, pneumocyte and fibroblastic hypertrophy and hyperplasia, edematous and degenerative changes, necrosis and airway epithelial denudation, atelectasis, hyperemia, hemorrhage, and exudation in the asthma group. Myrtenol, similar to budesonide, decreased inflammation and other abnormalities (×100 magnifications and scale bar, 200 μm). **a** Control. **b** Asthma. **c** Asthma/vehicle. **d** Asthma/myrtenol. **e** Asthma/budesonide. **f** Effects of myrtenol on pathological changes following asthma. Data are shown as means ± SEM. ***$p < 0.001$ versus CTL; ###$p < 0.001$ versus asthma.

Results

Effects of Myrtenol Inhalation on Anxiety-Like Behaviors of Asthmatic Rats in the Elevated plus Maze

The asthmatic rats revealed a significant reduction in time spent in the open arms compared to the control group. Conversely, myrtenol in asthma/myrtenol group showed a significant elevation in time spent in the open arms. The time spent in the closed arms by asthmatic rats was higher than healthy rats, but myrtenol inhalation reduced this parameter. Evaluating the number of head dips disclosed that this value significantly decreased in the asthma group compared to the control group, and myrtenol inhalation significantly raised head dips in the treatment group (Fig. 3).

Effects of Asthma and Myrtenol Inhalation on the Novel Objective Recognition Test

There was no significant difference between the groups in terms of the time spent around similar objects in the first trial (Fig. 4). In the test phase, our results showed that object recognition memory was impaired following asthma as manifested by spending less attention time to explore the novel object. Myrtenol inhalation improved the asthma-elicited disruption of the novel objective recognition test (Fig. 4).

Effects of Asthma and Myrtenol Administration on the Novel Location Recognition Test

There was no significant difference between the groups in terms of the time spent around objects in the first trial (Fig. 5). In the test phase, the rats in the control, asthma/

myrtenol, and asthma/budesonide groups spent significantly more time detecting the object in the novel place than the object in its original place. In contrast, the rats in the asthma group could not discriminate between the two locations compared with the control group (Fig. 5).

Effect of Myrtenol Administration on the Level of Cytokines in BALF

BALF cytokines were evaluated to confirm the asthma induction model in the animals. The levels of IL-10 in the rats with asthma were decreased compared to the control group; in the groups treated with myrtenol, the level of IL-10 was elevated compared to the asthma group. In the rats with asthma, the levels of TNF-α increased compared to the control group. However, with the inhalation of myrtenol, the levels of TNF-α were reduced compared with the asthma group (Fig. 6).

Assessment of Cytokines in the Hippocampus Tissue Supernatant

Analysis of hippocampus tissue sample suspensions disclosed that the levels of TNF-α, IL-6, and IL-17 were significantly higher in the asthmatic group compared to the control group. Furthermore, following myrtenol administration, levels of TNF-α, IL-6, and IL-17 significantly decreased compared to the asthmatic group. Moreover, the level of IL-10 as an anti-inflammatory cytokine was less in the asthma group than in the control group, and myrtenol inhalation increased IL-10 compared to the asthma group (Fig. 7).

Effect of Myrtenol Inhalation on Oxidative and Antioxidative Indices in the Hippocampus

The level of MDA in the hippocampus was elevated in the asthma group compared to the control group. Myrtenol reduced MDA levels compared to the asthma group. The levels of SOD activity, GPX activity, and TAC in the hippocampus decreased in the asthma group compared to the control group. Myrtenol elevated the levels of SOD activity, GPX activity, and TAC in comparison with the asthma group (Fig. 8).

Effect of Myrtenol Inhalation on Lung Pathological Changes in Asthmatic Rats

The hematoxylin/eosin staining of the lung tissue was performed to confirm the asthma induction model in the animals and to determine the optimum dose in the dose-response study. Our results showed that pathological changes in asthma groups were greater than in the control group, while myrtenol inhalation reduced pathological changes in the lung compared to the asthmatic rats (Fig. 9).

Discussion

Brain tissue is susceptible to changes in oxygen levels. Some brain insults, such as asthma, trauma, and stroke, might alter the oxygen supply to the brain [49–52]. Hypoxia could result in structural and functional irreversible brain damage [53–55]. Epidemiological studies have revealed that the risk of cognitive impairments is more than 78% in patients with asthma [15, 56]. Thus, finding a way to attenuate cognitive declines induced by asthma is necessary. Therefore, in the current research, we investigated the improvement impact of myrtenol (an alcohol monoterpene) on anxiety and cognitive impairments in asthmatic rats. The potential modulatory impact of myrtenol on inflammation and oxidative stress was also examined in the rat's hippocampus. Our findings demonstrated that myrtenol decreases asthma-induced anxiety-like behaviors and cognitive declines. It also has anti-inflammatory and antioxidant effects. Notably, these effects of myrtenol are similar to budesonide, which is a standard asthma treatment. Our findings revealed that following asthma the level of TNF-α increased and the level of IL-10 decreased in BALF of rats and myrtenol could reverse these changes. These data are consistent with our previous studies [31, 37] and showed that our asthma model induced successfully and myrtenol had anti-inflammatory effects in lung level.

Many clinical and experimental evaluations have reported that asthma induces both emotional and cognitive changes [17, 57, 58]. A higher prevalence of anxiety has been reported in patients with asthma and chronic obstructive pulmonary disease than in healthy individuals [59]. In the current study, we also observed anxiety-like behaviors in rats with asthma in the EPM test. These observations suggest that asthma may be a risk factor for the progression of anxiety-like behaviors, but its underlying neural basis is not yet well understood.

In another part of this research, we observed that asthma caused nonspatial and spatial memory deficits in the rats investigated in the NLT and NORT. In both of these tests, the discrimination ratio in the test phase decreased. These data represent an impaired function in memory tasks, consistent with previous investigations [17, 18]. Mokhtari-Zaer et al. [60] also observed spatial

learning and memory deficits in the MWM (Morris water maze), synaptic plasticity impairment, and a reduction in the BDNF level in the hippocampus of rats with chronic asthma. Some studies have also shown cognitive decline induced by asthma, and their results are consistent with our data [13, 14]. The precise underlying mechanisms of the impacts of asthma on the brain are unclear. Still, it has been found that both inflammation and oxidative stress are raised in asthma [61]. The current study also concluded that due to asthma, some antioxidants, including GPX, SOD, and TAC levels, were diminished, but the MDA (an oxidant) concentration was enhanced in the hippocampus of asthmatic rats compared to the control group. This finding is in line with the results of a previous investigation [14]. Chronic oxidative stress increases reactive oxygen species which are involved in anxiety and cognitive malfunction in humans and rodents [62, 63]. Consequently, in asthma, the oxidants-antioxidants balance is disrupted, thereby changing the production of inflammatory and anti-inflammatory cytokines. It has been revealed that systemic inflammation in asthma through elevation of IL-4 and IL-5 [64–66] can directly or indirectly result in brain damage [67]. Furthermore, an increase in IL-6, IL-1β, and TNF-α has been observed in the serum and cultured hippocampal neurons and lung cells of the fetal brain of asthmatic rats [68]. Neuronal injury by asthma occurs through increasing pro-inflammatory cytokines in the prefrontal cortex and hippocampus [69]. Our study also indicated an increase in the level of TNF-α, IL-6, and IL-17, and a reduction in the level of IL-10 in the hippocampus of rats with asthma, which is in line with previous studies [10, 11, 31, 70, 71]. Therefore, the results of the present investigation suggested that cognitive disturbances may be, in part, due to brain injury, which is caused by oxidative stress and neuroinflammation.

Myrtenol has anxiolytic, anti-oxidant, and anti-inflammatory impacts [29, 72], properties which make it a favorable compound to be utilized in research. Via its antioxidant activity, myrtenol can improve short-term memory deficits in the animal model of Parkinson's disease [73]. It can also reduce oxidative stress in the fibromyalgia model by decreasing the SOD/CAT ratio, which then prevents cognitive impairment and anxiety [32]. A previous study indicated that myrtenol attenuated inflammation incidences, changed the oxidant-antioxidant balance, and, therefore, could mitigate the impairments induced by asthma [37]. Viana et al. [74] also reported that myrtenol at oral doses of 25, 50, and 100 mg/kg significantly decreased the severity of ethanol-induced gastric lesions by oxidative stress modulation. The anti-inflammatory and antinociceptive effects of myrtenol in mice are demonstrated by Silva et al. [75]. In addition, the anxiolytic-like effect of myrtenol may be mediated by GABAergic transmission because myrtenol appears to improve GABA neurotransmission, in particular, via GABA(A) activation [29]. Myrtenol acts on δ subunit-containing GABA(A) receptors and enhances tonic inhibition in dentate gyrus granule cells [76]. The anxiolytic effects of myrtenol have been demonstrated in some tasks, including open field, plus maze, and light-dark compartment tasks [29, 77]. It seems that myrtenol's effects on CNS are driven by the ease with which it can cross the blood-cerebrospinal barrier [73, 78, 79].

Based on our previous research [31], in the current study, the impact of myrtenol on asthma-induced anxiety-like behaviors, cognitive declines, oxidative stress, and inflammation was evaluated in asthmatic rats. The findings disclosed that similar to budesonide, myrtenol had improvement effects on cognitive impairment and anxiety-like behaviors in this asthma model. Myrtenol as an anti-inflammatory agent decreased TNF-α, INL-6, and -17 (some inflammatory enzymes) and reduced IL-10 (a strong anti-inflammatory component). It also restored GPX, SOD, and CAT (important antioxidant enzymes) activities and diminished the MDA level in the hippocampus of asthmatic rats. Akefe et al. [80] reported that myrtenol mitigates streptozotocin-induced spatial memory deficit by improving oxidative stress and inflammation and modulating cholinergic and neurotransmitter functions in mice. Moreover, the neuroprotective effects of myrtenol are reported in Parkinson's disease and dementia models via modulating cholinergic and oxidative status [79, 81]. According to the cited evidence and the findings of the current research, it is concluded that the anti-inflammatory and antioxidant properties of myrtenol may underlie its effects against asthma-induced anxiety and cognitive impairments.

Conclusion

The present study showed that asthma exhibits deficient spatial and nonspatial memory, along with increased inflammation and oxidative stress in the rat hippocampus. However, these impacts were attenuated by myrtenol inhalation. This may provide a novel route to the remediation of cognitive impairments in pa-

tients with asthma, but effects of myrtenol on inflammation and oxidative stress in asthma merit more research.

Acknowledgments

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Statement of Ethics

Research ethics code is IR.KMU.REC.1399.603 that is approved in Kerman University of Medical Sciences, Kerman, Iran.

Conflict of Interest Statement

The authors have no relevant financial or nonfinancial interests to disclose.

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Author Contributions

Mohammad Abbas Bejeshk, Amir Hashem Aminizadeh, Elham Jafari, Akram Nezhadi, Faezeh Akhgarandouz, and Fatemeh Mohammadi: acquisition, analysis, or interpretation of data. Sina Motamedi, Iman Zangiabadi, Ahmad Ghasemi, Mazyar Fathi, Fatemeh Bejeshk, and Leila Mohammadi: drafting the work. Mohammad Amin Rajizadeh: conception or design of the work.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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