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Investigation of the Antiasthmatic Effects of Zufa Jewheri Used in Traditional Uyghur Medicine: An Experimental Study

Geleneksel Uygur Tıbbında Kullanılan Zufa Cevheri'nin Antiastmatik Etkilerinin Araştırılması: Deneysel Çalışma

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This study was presented orally 12th Acupuncture and Complementary Medicine Congress, September 15-17, 2023, Kocaeli, Türkiye.

ABSTRACT Objective: In this study, the effects of Zufa Jewheri, an aqueous extract of a herbal mixture used in Uyghur medicine, on ovalbumin-induced allergic asthma model in mice were investigated. Gas chromatography-mass spectrometry analysis was performed to detect fatty acids and monoterpenes present in the herbal mixture. **Material and Methods:** Ovalbumin-sensitized and -challenged BALB/c mice were orally administered varying doses of the herbal mixture and its aqueous extract. The effectiveness of the treatments was evaluated by Penh values, serum and bronchoalveolar lavage fluid (BALF) interleukin (IL)-4 and IL-5 levels, serum immunoglobulin E (IgE) levels and histological lung inflammation. **Results:** Ovalbumin-sensitized mice exhibited increased Penh values, elevated serum and BALF IL-4 and IL-5 levels, increased serum IgE levels and BALF eosinophil, neutrophil, lymphocyte and macrophage counts, and inflammation in lung tissue. Administration of 25 mg/kg of the herbal mixture (ZUFA-25) showed positive effects by significantly decreasing Penh values, serum and BALF IL-4 and IL-5 levels, BALF inflammatory cell counts and lung inflammation. Administration of 125 and 250 mg/kg of the mixture also reduced Penh values, serum and BALF IL-4 and IL-5 levels and inflammatory cell levels. **Conclusion:** This study confirms the traditional use of Zufa Jewheri for asthma. Zufa Jewheri Powder Mixture reduced airway hyperresponsiveness and inflammation in a mouse model of allergic asthma. These findings indicate the potential of Zufa Jewheri as adjunctive therapy for asthma. Further clinical studies in humans are needed to confirm its efficacy and safety.

Keywords: Uyghur medicine; traditional medicine; Zufa Jewheri; allergic asthma

ÖZET Amaç: Bu çalışmada, Uygur tıbbında kullanılan bitkisel bir karışımın sulu ekstraktı olan Zufa Cevheri'nin, farelerde ovalbümin ile indüklenen alerjik astım modeli üzerindeki etkileri araştırılmıştır. Gaz kromatografisi-kütle spektrometresi analizi ile bitkisel karışımdaki yağ asitleri ve monoterpenler tanımlanmıştır. **Gereç ve Yöntemler:** Ovalbümin ile duyarlılaştırılmış ve provoke edilmiş BALB/c farelerine bitkisel karışım ve sulu ekstre değişen dozlarda oral olarak uygulanmıştır. Tedavilerin etkinliği Penh değerleri, serum ve bronkoalveolar lavaj (BAL) sıvısı interlökin (IL)-4 ve IL-5 düzeyleri, serum immünglobulin E (IgE) seviyeleri ve histolojik akciğer inflamasyonu ile değerlendirilmiştir. **Bulgular:** Ovalbümin ile duyarlılaştırılan farelerde Penh değerlerinde, serum ve BAL sıvısı IL-4 ve IL-5 düzeylerinde, serum IgE seviyelerinde, BAL sıvısı eozinofil, nötrofil, lenfosit ve makrofaj sayılarında ve akciğer dokusu inflamasyon skorlarında artış görüldü. Bitkisel karışımın 25 mg/kg dozda uygulanması (ZUFA-25) Penh değerlerini, serum ve BAL sıvısı IL-4 ve IL-5 düzeylerini, BAL sıvısı inflamatuar hücre sayılarını ve akciğer dokusu inflamasyon skorunu anlamlı olarak azalttı. Karışımın 125 ve 250 mg/kg dozlarında uygulanması da Penh değerlerini, serum ve BALF IL-4 ve IL-5 seviyelerini ve inflamatuvar hücre düzeylerini azalttı. **Sonuç:** Bu çalışma, Zufa Cevheri'nin astım için geleneksel kullanımını doğrulamaktadır. Zufa Cevheri Toz Karışımı, alerjik astım fare modelinde solunum yolu aşırı duyarlılığını ve inflamasyonunu azaltmıştır. Bu bulgular Zufa Cevheri'nin astım için yardımcı tedavi potansiyeline işaret etmektedir. Etkinliğini ve güvenliğini doğrulamak için insanlarda daha fazla klinik çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Uygur tıbbı; geleneksel tıp; Zufa Cevheri; alerjik astım

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Asthma, a chronic inflammatory condition of the airways, encompasses a range of cellular actors, such as mast cells, eosinophils, T lymphocytes, macrophages, neutrophils, and epithelial cells. The cause of asthma is not fully understood but may involve genetic factors, environmental triggers, and allergic reactions.[1]

Conventional asthma management usually relies on corticosteroids and short-acting β2-agonists administered at different dosages. Nevertheless, these antiasthmatic medications are associated with potential side effects including convulsions, cardiovascular issues, osteoporosis, myopathy, and growth impairment. Thus, the development of herbal products devoid of such adverse effects is seen as a significant stride in asthma care.[2]

Traditional Uyghur medicine uses a herbal mixture known as "Zufa Jewheri" for the treatment of asthma. The mixture consists of nine different plants, including *Foeniculum vulgare*, *Hyssopus officinalis* L, *Adiantum capillus-venesis*, *Ruta graviolens*, *Glycyrrhiza glabra*, *Apium graveolens*, *Rosa domescana*, *Urtica dioica* and *Trigonella foenum-gracum*.[3,4] Previous studies have shown that these plants have anti-inflammatory and bronchodilatory effects. Wang et al. found that *Hyssopus officinalis* exerts an anti-inflammatory effect, possibly by differentiating at the transcriptional level of Th1, Th2 and Th17.[5] Zemmouri et al. reported that *Urtica dioica* extract reduced serum leukocyte and lymphocyte levels and bronchoalveolar lavage fluid (BALF) eosinophils and suppressed inflammatory cell migration.[6] *Trigonella foenum-graecum* has been proposed to diminish the expression of Th2 cytokines, elevate Th1 cytokines in BALF and homogenated lung tissues, and demonstrate notable suppression of serum immunoglobulin E (IgE) and anti-OVA IgG1, potentially mitigating asthma episodes.[7]

In this research, we explored the anti-asthmatic properties of Zufa Jewheri, a traditional Uyghur medicinal herb for treating asthma, through a mouse model of allergic asthma. Comprised of nine plants with known anti-inflammatory and bronchodilatory properties. Through this investigation, we aim to provide a fundamental basis for the potential therapeutic benefits of Zufa Jewheri in the management of asthma, offering an alternative approach that may avoid the side effects commonly associated with conventional antiasthmatic drugs.

MATERIAL AND METHODS

ANIMAL GROUPS AND SOURCE OF PLANTS MATERIAL

Ethics committee approval was obtained, and all experimental protocols were conducted at the Verification Experimental Application and Research Laboratory of Ankara Technical University (date: September 29, 2022; no. KN.0016/2022). The Research Council, USA procedures followed the guidelines outlined in the Guide for Care and Use of Laboratory Animals by the National, and adhered to the principles of the Helsinki Declaration. BALB/c mice were utilized and housed under controlled conditions, maintaining a 12-hour light/dark cycle at a room temperature of 22 °C, with access to standard chow and water *ad libitum*.

Zufa Jewheri was prepared according to the prescribed method mentioned in the traditional Uygur medicine book called "Dori Ulqimi".[3] The nine plants included in Zufa Jewheri (*Foeniculum vulgare*, *Hyssopus officinalis* L, *Adiantum capillus*, *Ruta graviolens*, *Glycyrrhiza glabra*, *Apium graveolens*, *Rosa Domestica*, *Urtica dioica*, *Trigonella goenum-gracum*) were obtained from local herbal markets. Zufa Jewheri was obtained from Chinese herbal markets.

GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS

Zufa Jewheri subjected to gas chromatography-mass spectrometry (GC-MS) analysis. However, the expected compounds were not obtained. Considering that this may be due to the large removal of water by heat during the preparation process, an aqueous extract of Zufa Jewheri Aqueous Extract (ZJAE) was prepared without complete removal of water (1:9 ratio) in order to preserve the components, and analyzed by GC-MS. Furthermore, a powder mixture of Zufa Jewheri Powder Mixture (ZJPM) was prepared. Ethanol and hexane extracts of this mixture were obtained and subjected to GC-MS analysis. The hexane extract yielded a higher number and proportion of components. Subsequently, the hexane extract of the

powder mixture yielded enhanced results. Therefore, ZJPM suspended in corn oil was administered to the animals.

The analysis of the extracts was carried out using GC-MS (Trace 1300, TSQ Duo) with a capillary TG-5MS column (Thermo Scientific, USA) and electron impact mode. The extracts were injected into the system with an injection volume of 1 µL using an automated injector module. The injection was performed in splitless mode. The extracts were qualitatively analyzed in full scan mode within the 40-550 (m/z) range at an electron energy of 70 eV. The temperature was held at 60 °C for 3 minutes, then increased to 280 °C at a rate of 5 °C/min, and maintained at 280 °C for 25 minutes. The carrier gas (helium) flow rate was set at 1 mL/min. The NIST and Wiley GC-MS Compound Search Libraries were utilized for the analyses.

ACUTE TOXICITY TEST

Acute toxicity tests were performed on 6 animals prior to the study. Following the acute toxicity testing protocol, 2 animals were administered ZJAE at doses of 5000, 10000 and 15000 mg/kg and followed for 24 hours. The animals were observed for heart rate, respiration, body temperature, reflex movements and general condition.[8]

ALLERGIC ASTHMA MODEL

Allergic asthma was induced in mice using a previously described method.[9] On days 0, 14 and 21, mice were sensitised with 1 mg/kg ovalbumin (Grade V) and 100 mg/kg aluminium hydroxide prepared in saline, administered intraperitoneally (i.p.). On days 28, 29 and 30 of the experiment, each group was exposed to inhalation of a 1% ovalbumin solution for 30 min via a nebulizer (0.5 mL/min).

EXPERIMENTAL PROTOCOL

Dora Ulqumi states that the recommended dose of Zufa Jewheri for adults is 12 g, which corresponds to a dose of 171 mg/kg to be taken twice a day (morning and evening). Using the equation "Human Dose (mg/kg)=Animal Dose (mg/kg) x (Animal Km/Human Km)", the dose of Zufa Jewheri aqueous form for 40 g mice was approximately 2 g/kg.[3,10] There is no prior in vivo study on ZJPM. Therefore, we used the results of the GC-MS analysis of the ZJAE and ZJPM's hexane extract to determine the dosage of ZJPM. We decided to administer ZJPM at doses of 25, 125, and 250 mg/kg based on the peak areas of the components commonly detected in both ZJAE and hexane extract of ZJPM.

Mice were divided into 10 groups (n=7): Control, OVA, ZUFA-25 (25 mg/kg ZJPM), ZUFA-125 (125 mg/kg ZJPM), ZUFA-250 (250 mg/kg ZJPM), ZUFA-AE (2000 mg/kg ZJAE).

The animals received intraperitoneal injections of 1 mg/kg ovalbumin and 100 mg/kg Al(OH)3 on days 0, 14, and 21 to induce allergic asthma in mice. On days 28-30, the mice were treated with 1% ovalbumin solution using a nebuliser for 20 min. The control group of mice remained untreated, while the treatment groups received intraperitoneal injections of ZJPM (25, 125, 250 mg/kg) and ZJAE (2000 mg/kg) throughout the study period (days 0-30) (Figure 1).

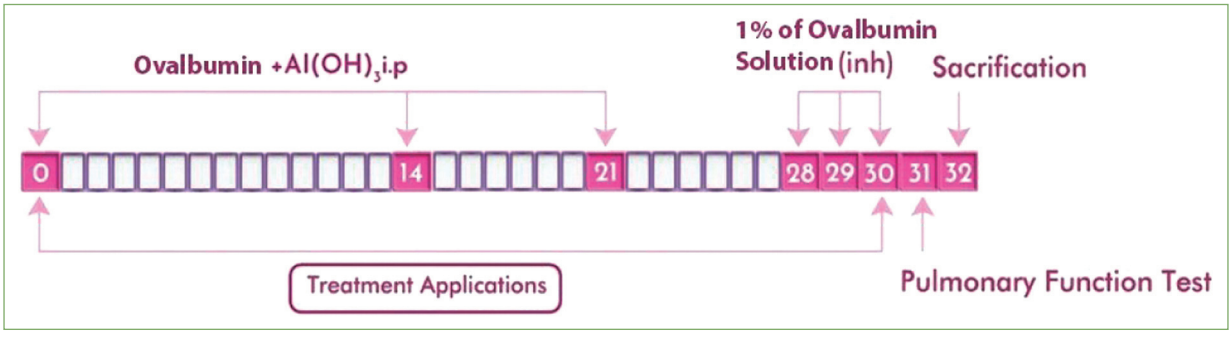

FIGURE 1: Experimental protocol.

PULMONARY FUNCTION TEST

In a setup involving conscious, unrestrained mice, Penh measurements were conducted 24 hours following the last ovalbumin challenge, utilizing whole body plethysmography (WBP) technology provided by Emka Technologies, Paris, France. The IOX2 software (Emka Technologies) is utilized to compute Penh values. These values signal alterations in the respiratory pattern and indicate bronchoconstriction when they exceed 1.[11] Mice were placed in the WBP enclosure where they were allowed a 10-minute acclimatisation period. To establish baseline Penh values, phosphate buffered saline (PBS) was aerosolized for three minutes. Subsequently, methacholine (MCh) was aerosolized at escalating concentrations (from 3.125 to 50 mg/kg). Penh values recorded over three minutes for each MCh concentration were then averaged. Airway hyperresponsiveness was quantified by calculating the percentage deviation from baseline Penh values.

COLLECTION OF BALF AND MEASUREMENT OF CYTOKINES

Following respiratory tests, ketamine (100 mg/kg) and xylazine (10 mg/kg) is given i.p. to induce anesthesia. It is followed by tracheostomy and tracheal cannulation. The left bronchus was ligated, and the lungs were lavaged with 0.5 mL PBS three times. After centrifugation of the collected BALF, IL-4 and IL-5 levels were measured by ELISA.

MEASUREMENT OF SERUM IL-4, IL-5 AND IGE LEVELS

Blood was collected from the mice's hearts and centrifuged. Serum concentrations of IL-4, IL-5, and IgE were determined using ELISA kits as per manufacturer guidelines.

INFLAMMATORY CELL COUNT IN BALF

The BALF pellet was suspended in saline solution, spread onto slides, and then stained with hematoxylin and eosin. The percentages of eosinophils, neutrophils, lymphocytes, and macrophages were quantified by examining 100 cells per slide.

HISTOPATHOLOGICAL EXAMINATION OF LUNG TISSUE

Mice were euthanized, and their lungs were fixed in 10% formalin solution, followed by histological processing of randomly selected paraffin-embedded sections. Following deparaffinization, they were stained with hematoxylin and eosin. Inflammation levels in lung tissues were semi-quantitatively assessed using the following scoring system: 0 for no inflammation, 1 for mild, 2 for moderate, and 3 for severe cellular infiltration.

STATISTICAL ANALYSIS

Data were analyzed using IBM SPSS software (United States). Parametric data were analyzed with Student's t-test and one-way analysis of variance, while non-parametric data were analyzed with the Mann-Whitney U and Kruskal-Wallis tests. Statistical significance is accepted as below $p<0.05$.

RESULTS

GC-MS ANALYSIS

GC-MS analysis results showed that water dissolved form of Zufa Jewheri contained 10 components (results not shown), whereas ZJAE contained 20 components (Table 1). Ethanol and hexane extracts of ZJPM contained 18 (results not shown) and 50 components, respectively (Table 2).

In our study, when GC-MS results of ZJAE and hexane extracts of ZJPM administered to mice were compared, estragole, hexadecadienoic acid methyl ester, 9-octadecenoic acid, 9,12-octadecadienoic acid (z,z)-, 2,3-bis[(trimethylsilyl)oxy]propyl ester, heptacosane, isochiapin b, dotriacontane, 9,12,15-octadecatrienoic acid and 2-[(trimethylsilyl) oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester were identified as common compounds.

ACUTE TOXICITY TEST

Mice treated with ZJAE were monitored for 24 hours. Those receiving 5000 mg/kg exhibited high activity for the initial 8 hours, followed by a period of calmness lasting approximately 3 hours, after which normal behavior resumed. Mice given doses of 10000 mg/kg and 15000 mg/kg showed reduced activity for

TABLE 1: GC-MS analysis of ZJAE.

Peak no	RT time	Component name	Probability	Area (%)
1	8.41	7,7-Bis(methylthio)-6-methyl-1-(2-thienyl)-2,4,6-heptatriien-1-one	24.33	8,078588653
2	17.18	Estragole	30.35	2,647899342
3	26.37	6-Butyl-1,4-cycloheptadiene	13.56	1,062817363
4	26.43	4-Ethylbenzoic acid, allyl ester	11.63	0,726891115
5	27.02	2H-Inden-2-one, 1,4,5,6,7,7a-hexahydro-4-methyl-7-(1-methylethyl)- (CAS)	12.09	0,338275551
6	31.9	Pentadecanoic acid, 14-methyl-, methyl ester	32.01	4,794410089
7	32.7	Quercetin 7,3',4'-Trimethoxy	35.86	0,122111289
8	35.13	Hexadecadienoic acid, methyl ester	18.41	1,484148379
9	35.35	9-Octadecenoic acid, (Z)-	11.95	0,138500065
10	35.69	Octadecanoic acid, methyl ester	33.83	3,664189417
12	36.52	Decanedioic acid, dibutyl ester	95.39	39,05140704
13	42.58	9,12-Octadecadienoic acid (Z,Z)-, 2,3-bis[(trimethylsilyl)oxy]propyl ester (CAS)	27.74	0,077012947
14	44.89	Docosane	13.77	3,094657469
15	45.70	1,2-Benzenedicarboxylic acid, dioctyl ester (CAS)	22.26	9,537518724
16	47.7	Heptacosane	23.4	2,973822624
17	48.26	Isochiapin B	3.99	0,105369603
18	51	10-Nonadecanone	57.42	21,95032673
19	51.15	Dotriacontane (CAS)	18.34	0,125633071
20	53.86	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)-	17.53	0,026420528

GC-MS: Gas chromatography-mass spectrometry; ZJAE: Zufa Jewheri Aqueous Extract.

8 hours before returning to normal behavior. No fatalities were recorded.

PENH VALUES

In ovalbumin-sensitive mice, Penh values exhibited a notable increase compared to control mice at MCh concentrations ranging from 6.25 to 50 mg/mL ($p<0.05$). The treatment groups (ZUFA-25, ZUFA-125, ZUFA-AE) displayed significantly decreased Penh values compared to the OVA group at all MCh concentrations ($p<0.05$). ZUFA-250 exhibited significantly lower Penh values than OVA at 12.5, 25, and 50 mg/mL ($p<0.05$). There were no significant differences in Penh values between the ZUFA-25 and ZUFA-AE groups across all concentrations (Figure 2).

SERUM IL-4 AND IL-5 LEVELS

Serum levels of IL-4 and IL-5 were markedly elevated in the OVA group compared to control ($p<0.05$). In the ZUFA-25 group, IL-4 levels were significantly reduced compared to OVA ($p<0.05$), and they were even lower compared to ZUFA-AE ($p<0.05$). Regarding IL-5, the ZUFA-25, ZUFA-125, ZUFA-250, and ZUFA-AE groups exhibited significantly lower levels compared to OVA ($p<0.05$). There was no significant difference in IL-5 levels observed between the control and ZUFA-25 groups ($p>0.05$) (Figure 3).

BALF IL-4 AND IL-5 LEVELS

In OVA-sensitized and challenged mice, BALF IL-4 and IL-5 levels were found higher compared to controls ($p<0.05$). The ZUFA-25 group exhibited significantly lower BALF IL-4 levels compared to OVA ($p<0.05$), with no significant difference compared to controls ($p>0.05$). BALF IL-5 levels were lower in the ZUFA-25 and ZUFA-125 groups compared to OVA ($p<0.05$), with no significant difference compared to controls ($p>0.05$). Additionally, the ZUFA-25 group showed lower BALF IL-5 levels compared to ($p<0.05$) (Figure 4).

SERUM IGE LEVELS

Serum IgE levels of mice were higher in the OVA group than the control group ($p<0.05$). No significant changes were observed in, ZUFA-25, ZUFA-125, ZUFA-250, and ZUFA-AE groups compared to the OVA group (Data not shown.).

TABLE 2: GC-MS analysis of the hexane extract of the ZJPM.

Peak no	Real time	Component name	Probability	Area (%)
1	4.21	(3R)-3-Phenyl-2,3-dihidro-1H-isoindol-1-one	41.23	0,004198
2	6.88	Bicyclo[3,1,1]hept-2-ene, 2,6,6-trimethyl-, (ñ)-	21,33	0,028423
3	8.06	2-α- pinene	37.35	0,026924
4	8.41	α -Myrcene	52.57	0,053539
5	8.85	Sabinene	20.95	0,000994
6	9.63	dl-Limonene	17.08	6,560813
7	11.36	Fenchone	40.18	0,159505
8	12.43	Trans-p-Mentha-2,8-dienol	41.11	0,003361
9	12.54	α-Ocimene-X	22.97	0,001173
10	12.86	p-mentha-E-2,8(9)-dien-1-ol	12.86	0,013534
11	14.66	Estragole	64.57	0,690062
12	15.38	Trans-(+)-Carveol	39.8	0,002172
13	17.26	Trans Anethole	33.08	11,37636
15	18.92	1,2-Cyclohexanediol, 1-methyl-4-(1-methylethenyl)- (CAS)	56.95	0,056027
16	19.06	α-Chlorobutyrophenone	14.20	0,007889
17	19.72	2,3-Dimethyl-para-anisaldehyde	10.38	0,533111
18	19.99	Geranyl isovalerate	20.88	0,003494
19	20.73	Trans-Caryophyllene	23.38	0,003022
20	22.5	α-Selinene	23.77	2,403578
22	23.02	Butyl Hydroxy Toluene	66.96	1,164485
23	24.83	(-)-Caryophyllene oxide	74.79	0,008819
24	27.13	2H-Inden-2-one, 1,4,5,6,7,7a-hexahydro-4-methyl-7-(1-methylethyl)- (CAS)	36.68	2,562676
25	27.82	Trans-Z-à-Bisabolene epoxide	14.58	0,003756
27	28.21	2-propenyl phenoxyacetate	15.89	6,248412
28	28.36	Ligustilide	14.5	0,00951
29	30.14	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	25.63	0,00965
30	30.85	Thiosulfuric acid (H2S2O3), S-(2-aminoethyl) ester	28.2	0,002373
31	31.34	7-Methyl-Z-tetradecen-1-ol acetate	13.74	0,002794
33	33.28	Hexadecanoic acid	59.88	0,694317
34	36.62	9,12-Octadecadienoic acid (Z,Z)-	26.33	32,18658
35	38.69	Docosanoic acid (CAS)	11.92	3,050429
36	40.74	9,12-Octadecadienoyl chloride, (Z,Z)-	13.09	0,122099
37	41.73	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	22.71	13,18384
38	42.03	9-Octadecenoic acid (Z)- (CAS)	24.02	0,053186
39	43.45	Docosane (CAS)	7.29	0,124981
40	44.32	Hi-oleic safflower oil (CAS)	34.15	0,017697
41	44.96	Heptacosane (CAS)	17.55	2,638038
42	45.07	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	10.97	0,060302
43	46.76	1-Heptatriacotanol	13.7	0,01421
44	47.18	Dotriacontane (CAS)	47.18	0,034469
45	47.33	Isochiapin B	10.2	0,020901
46	47.88	Hentriacontane	11.4	7,59627
47	48.74	Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy-	17.04	0,080106
48	49.51	Tetratetracontane (CAS)	16.29	6,384576
49	51.02	2,2-Dideutero Octadecanal	22.15	0,020658
50	51.42	Pentatriacontane (CAS)	12.11	1,776687

GC-MS: Gas chromatography-Mass spectrometry; ZJPM: Zufa Jewheri Powder Mixture.

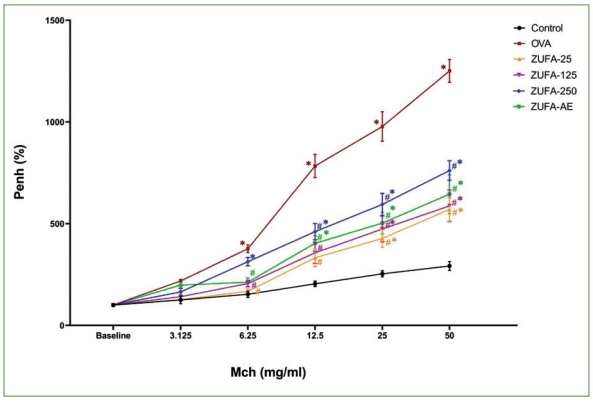

FIGURE 2: Penh values.

BALF EOSINOPHIL, NEUTROPHIL, LYMPHOCYTE AND MACROPHAGE LEVELS

In OVA-sensitized and challenged mice, BALF levels of eosinophils, neutrophils, lymphocytes, and macrophages were increased compared to controls ($p<0.05$). However, in the ZUFA-25 group, these values were less than those of the OVA group. ($p<0.05$). Comparing with controls, ZUFA-25 group showed no significant difference in eosinophil and lymphocyte levels ($p>0.05$), but higher levels of neutrophils and macrophages ($p<0.05$).

In the ZUFA-125 group, neutrophil and lymphocyte levels were lower compared to OVA ($p<0.05$), however no significant differences were found in eosinophil and macrophage levels ($p>0.05$). Compared to controls, ZUFA-125 group showed higher levels of neutrophils, lymphocytes, and macrophages ($p<0.05$), but not in eosinophil levels ($p>0.05$).

ZUFA-250 group displayed reduced lymphocyte levels compared to OVA ($p<0.05$), however no significant differences were found in eosinophil, neutrophil, and macrophage levels ($p>0.05$). Compared to controls, the ZUFA-250 group exhibited higher levels of lymphocytes and macrophages ($p<0.05$), with no significant differences in eosinophil and neutrophil levels ($p>0.05$).

Comparing ZUFA-25 and ZUFA-AE groups, no significant difference was observed in eosinophil levels ($p>0.05$), but ZUFA-25 group showed lower levels of neutrophils, lymphocytes, and macrophages ($p<0.05$) (Figure 5).

HISTOPATHOLOGICAL EXAMINATION OF LUNG TISSUE

In mice from the OVA group, lung tissue inflammation scores were notably elevated compared to controls ($p<0.05$). However, in the ZUFA-25 group, inflammation scores were lower than those in the OVA group ($p<0.05$). Inflammation scores of treatment groups and the OVA group were similar ($p>0.05$) (Figure 6).

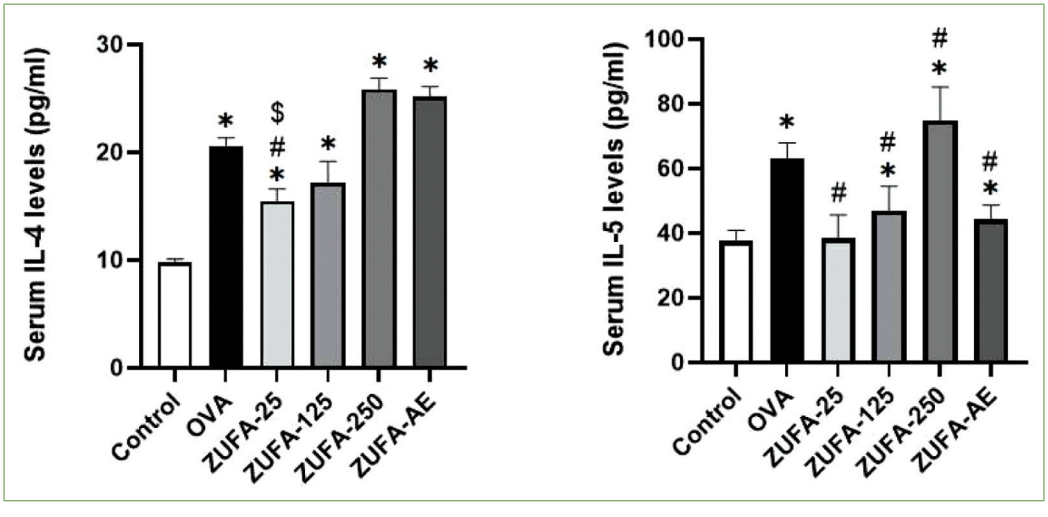

FIGURE 3: Serum levels of IL-4 and IL-5.

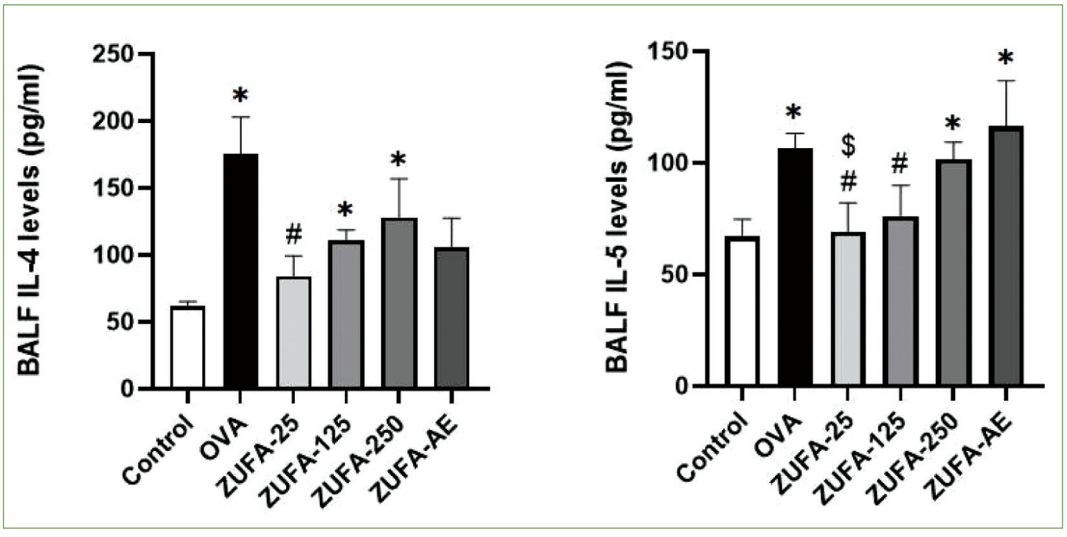

FIGURE 4: BALF levels of IL-4 and IL-5.

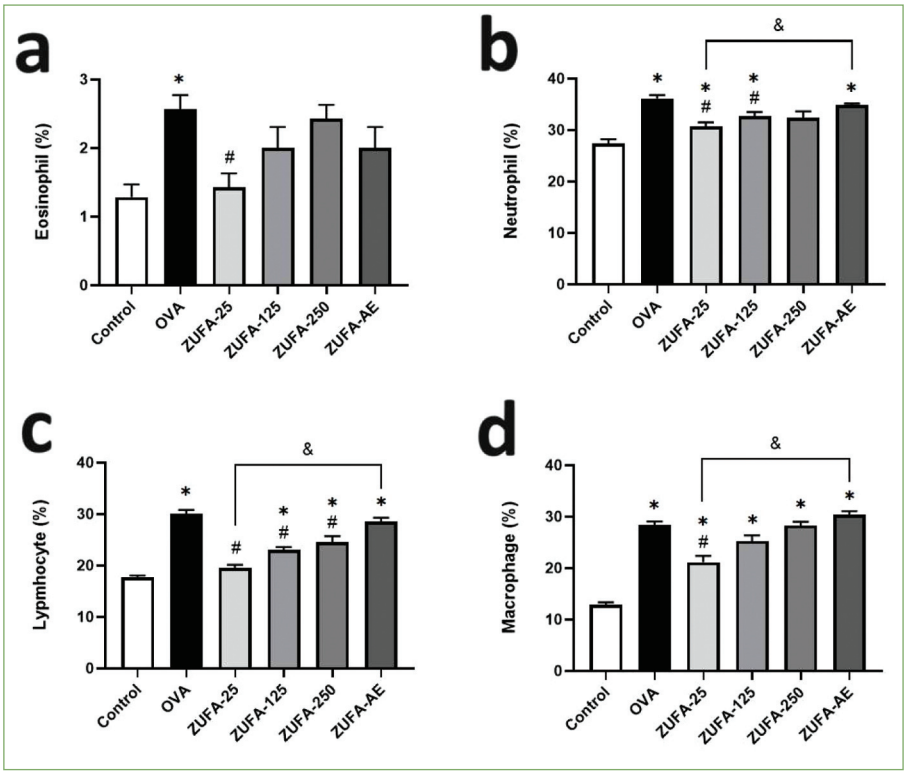

FIGURE 5: BALF inflammatory cell levels.

DISCUSSION

Zufa Jewheri is traditionally used in East Turkestan for treating respiratory diseases such as cough and asthma caused by cold-induced nasal congestion.[12] Zufa Jewheri's formulation contains 9 plants, namely *Foeniculum vulgare*, *Hyssopus officinalis* L, *Adiantum capillus-venesis*, *Ruta graviolens*, *Glycyrrhiza glabra*, *Apium graveolens*, *Rosa domescana*, *Urtica dioica*, and *Trigonella foenum-graecum*. Pharmacological research into the anti-asthmatic potential of plants identifies *Adianthum capillus-veneris*,

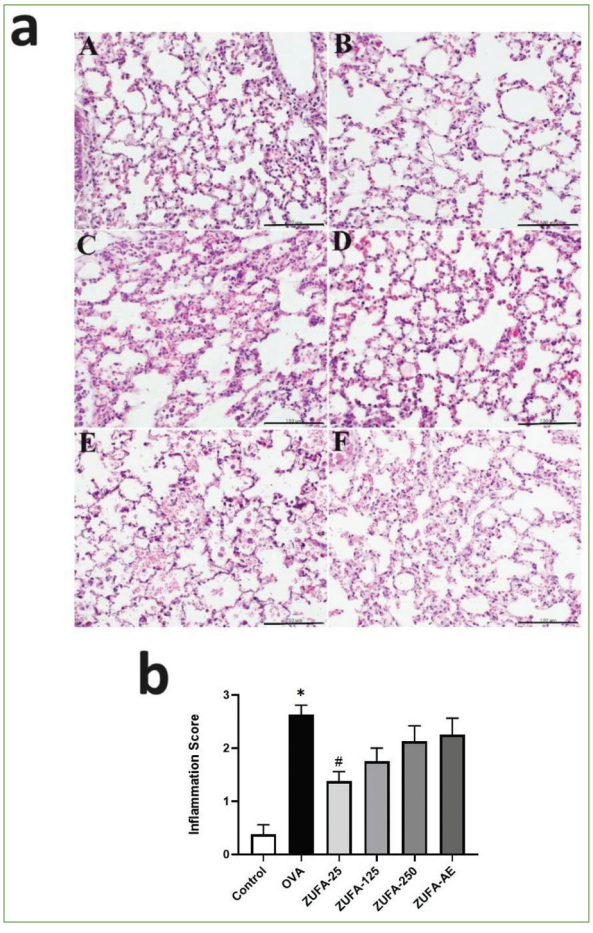

FIGURE 6: Lung tissues stained with hematoxylin and eosin, 400x magnification (a). Inflammation scores in the lung tissue (b).

Boswellia oleogum resin, *Crocus sativus*, *Glycyrrhiza glabra*, *Hyssopus officinalis*, and *Ruta graveolens* as the most potent botanical treatments for asthma. These findings are corroborated by studies that have shown these plants' efficacy in modulating inflammation, oxidative stress, allergic responses, constriction of tracheal smooth muscles, and remodeling of the airways.[13] In recent times, notable progress has been achieved in diagnosing and treating asthma, accompanied by the development of extensive and commonly employed clinical guidelines for its management. Nonetheless, achieving complete control over asthma continues to pose challenges.

In our study investigating Zufa Jewheri's effects on asthma using a mouse model, we conducted GC-MS analyses on its water-soluble form (ZJAE) and hexane extract (ZJPM). Limited transfer of active compounds into the aqueous phase was observed in ZJAE, while ZJPM's hexane extract showed the highest component detection. Hence, ZJPM suspended in corn oil at 3 different doses and ZJAE at a single dose were orally administered to ovalbumin-sensitised mice to induce allergic asthma. Evaluation included Penh values, IL-4 and IL-5 levels in serum and BALF, serum IgE levels, inflammatory cell levels in BALF, and lung tissue histopathology. Our findings demonstrate that oral administration of ZJPM at 25 mg/kg effectively reduces airway hypersensitivity and inflammation, exerting anti-asthma effects.

Airway hyperresponsiveness, an important feature of asthma, occurs as a result of inflammation.[14] Penh value is a parameter related to airway resistance and can be determined by whole-body plethysmography.[15] In our study, Penh values in BALB/c mice with ovalbumin-induced allergic asthma were significantly elevated at all MCh concentrations compared to the control group. Treatment with all doses of ZJPM and ZJAE led to notable reductions in Penh levels compared to the disease group, with the most significant decrease observed in the ZJPM group receiving the lowest dose, ZUFA-25.

Allergic asthma is associated with Th2 cell-mediated immune responses. Upon exposure to the allergen, antigen-specific Th2 cells induce the production of type 2 cytokines (IL-4, IL-5, IL-9 and IL-13), leading to a substantial accumulation of eosinophils in the airways, while antigen-specific B cells produce IgE antibodies.[16] The development, maturation, recruitment and survival of eosinophils are significantly influenced by IL-5. Specific B cells are induced by IL-4 and IL-13 to produce IgE, which causes basophils and mast cells to degranulate and release pro-inflammatory mediators, breaking the barrier and remodelling tissue.[16-18] Some components of Zufa Jewheri have been studied to assess their anti-asthmatic effects. For instance, *Hyssopus officinalis* aqueous extract has shown to modulate Th1, Th2, and Th17 cell differentiation.[19] *Glycyrrhiza glabra* extract and its main component, glycyrrhizin, have been found to reduce, respectively, BALF levels of IL-5, IL-13, interferon-γ and serum IL-4, IL-5, and serum IgE in mice with ovalbumin-induced allergic asthma.[20,21] In our study, ZUFA-25 group has lower

IL-4, IL-5 levels of serum and BALF, and serum IgE compared to the disease group. ZUFA-125 group also showed lower levels of IL-5 in serum and BALF compared to the OVA group. Although not statistically significant, a trend of reduced IgE values in the ZUFA-25 group may be attributed to reduced IL-4 levels, which are involved in IgE production. Additionally, compared to the ZUFA-AE group, the ZUFA-25 group displayed lower levels of serum IL-4 and BALF IL-5. The reduction in Th2 cytokines in the ZUFA-25 group may contribute to the decrease in Penh levels.

In asthma and COPD, inflammatory cells such as eosinophils, neutrophils, macrophages, and lymphocytes migrate to the lungs, driven by chemotactic factors. These cells, along with structural lung cells like epithelial and endothelial cells, contribute to the inflammatory response.[22] Eosinophils play a major role in asthma, leading to airway dysfunction and tissue remodeling.[23] Severe asthma is linked to higher neutrophil counts and corticosteroid resistance.[24] CD4+ T cells are critical in allergic asthma, influencing IgE production by B cells.[25] Macrophages, derived from blood monocytes, show a mix of proinflammatory and anti-inflammatory activities.[26] In a study conducted by Dogan et al., using mice in an ovalbumin-induced allergic asthma model, it was observed that the main flavonoid compound, glabradin, found in *Glycyrrhiza glabra*, contained in Zufa Jewheri, led to a significant decrease in neutrophil, lymphocyte, eosinophil, and monocyte cell levels in BALF.[27] Piao et al. reported that *Trigonella gracum-foenum* extract reduced lymphocyte, eosinophil, neutrophil, and macrophage cell levels in BALF of mice with ovalbumin-induced allergic asthma.[7] In our study, the OVA group showed significantly elevated levels of eosinophils, neutrophils, lymphocytes, and macrophages compared to controls. Treatment with ZUFA-25 reduced these inflammatory cells to near control levels, particularly eosinophils and lymphocytes. The ZUFA-125 and ZUFA-250 groups also showed reductions in certain inflammatory cells, but ZJAE had no significant effect. The reduction in eosinophils in the ZUFA-25 group is likely linked to decreased IL-5 levels, which influence eosinophil migration to the lungs.

Airway remodeling in asthma involves structural changes like goblet cell metaplasia, increased subepithelial matrix proteins, fibrosis, angiogenic factor overexpression, and airway smooth muscle changes, impacting airway function.[28] It has been suggested that *Hyssopus officinalis* L. can regulate the MMP-9/TIMP-1 ratio and inhibit smooth muscle thickening, goblet cell hyperplasia, and fibrosis.[29] The main component of *Glycyrrhiza glabra*, glycyrrhizin, has been shown to have beneficial effects on long-term histopathological changes in the lungs, including basal membrane, epithelium, smooth muscle layer of the airways, goblet cell, and mast cell numbers in an asthma animal model.[30] *Trigonella foenum-graecum* extract was reported to decrease goblet cell numbers, eosinophil infiltration, and collagen accumulation in the bronchi of mice with an asthma model.[7] In our research, lung tissue inflammation scores were lower in treatment groups than in the disease group, with a significant reduction observed in the ZUFA-25 group. This decrease aligns with reduced cytokine levels in BALF and serum, and diminished inflammatory cell migration to lung tissue.

In our GC-MS analysis of the hexane extract of ZJPM, it was determined that 9-Octadecenoic acid (oleic acid), 9,12-Octadecadienoic acid (linoleic acid), 9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester, 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, docosanoic acid and hexadecanoic acid are abundant. In a study indicated that oleic acid reduced inflammatory cell infiltration into BALF, airway hypersensitivity, IL-5 levels, and serum IgE levels in mice with ovalbumin-induced allergic asthma.[31] Monoterpenes, which confer aromatic taste to certain plants, fruits, and vegetables with their volatile properties, are known to possess anti-inflammatory, analgesic, and antibacterial effects.[32] In our GC-MS analysis, we detected several monoterpenes, including trans-anethole, l-limonene, fenchone, α-pinene, sabinene, α-ocimene, isochiapin B, and trans-carveol. It was observed that trans-anethole reduced pulmonary eosinophilic infiltration, IL-4, IL-5, and IgE levels, and excessive mucus secretion in mice with ovalbumin-induced respiratory hypersensitivity.[33] The findings of these studies conducted on the components we detected in our GC-MS

analysis support the anti-asthmatic effect of the Zufa Jewheri.

Our study confirms the in vivo antiasthmatic efficacy of ZJPM and validates the traditional use of Zufa Jewheri in Uyghur Medicine. Through GC-MS analysis, we identified bioactive components known for their effectiveness in asthma. While the aqueous extract is traditionally preferred, our analysis revealed the hexane extract of the powder mixture to be richer in bioactive compounds. In our study, the administration of ZJPM at the lowest dose of 25 mg/kg demonstrated a therapeutic effect on asthma by reducing Penh values, serum and BALF IL-4 and IL-5 levels, inflammatory cell levels in BALF, and lung tissue inflammation. The ZJAE only resulted in a significant decrease in Penh values and serum IL-5 levels. According to the results, the powder mixture was more effective than the aqueous extract in our in vivo study. The finding that the lowest dose of the Zufa Jewheri powder mixture was more effective compared to the other doses is noteworthy. This may indicate a decrease in efficacy with increasing doses. However, further studies using different doses are needed to elucidate this issue.

CONCLUSION

In conclusion, our study provides scientific evidence supporting the traditional use of Zufa Jewheri in the treatment of asthma. Using an allergic asthma mouse model, the study shows that ZJPM significantly reduces airway hyperresponsiveness, airway inflammation and Th2-mediated immune responses. These findings suggest that Zufa Jewheri shows promise as an adjunctive treatment for asthma. The encouraging results highlight the need for further research, including clinical trials in humans, to confirm the efficacy and safety of Zufa Jewheri and optimize its therapeutic application.

Highlights of the Findings and Novelties

- Zufa Jewheri ameliorates airway inflammation and hyperresponsiveness in a mouse model of allergic asthma.
- The hexane extract of Zufa Jewheri contains a greater number of components compared to the aqueous extracts.
- The lower dose of Zufa Jevheri demonstrated superior anti-asthmatic activity.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Fidan Pesen Özdoğan, Fatma Uysal;* ***Design:*** *Fatma Uysal, Saliha Ayşenur Çam Özünlü;* ***Control/Supervision:*** *Seyfullah Oktay Arslan, Mağfiret Abdulveli Bozlar;* ***Data Collection and/or Processing:*** *Fidan Pesen Özdoğan, Fatma Uysal;* ***Analysis and/or Interpretation:*** *Fatma Uysal, Gülben Akcan;* ***Literature Review:*** *Fidan Pesen Özdoğan, Fatma Uysal;* ***Writing the Article:*** *Fidan Pesen Özdoğan, Fatma Uysal;* ***Critical Review:*** *Seyfullah Oktay Arslan, Mağfiret Abdulveli Bozlar;* ***References and Fundings:*** *Fidan Pesen Özdoğan, Fatma Uysal;* ***Materials:*** *Fidan Pesen Özdoğan.*

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