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Immunomodulatory Effect of SINA 1.2 Therapy Protocol in Asthmatic Mice Model: A Combination of Oxymel and Sauna

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ABSTRACT

Alternative medicine has become popular in asthmatic patients. We evaluated the immunomodulatory effects of the SINA 1.2 therapy protocol derived from Persian medicine in an asthmatic mice model.

Forty-two male BALB/c mice were divided into six groups: one control (sham) and five sensitized groups (by parenteral injection of 20 µg ovalbumin in 100 µL normal saline plus 50 µL alum on days 1 and 14). Sensitized groups were as: untreated, budesonide (1 mg nebulized budesonide: 200 µg/puff every 5 min for 25 min), dry sauna (30 min, 37°C), oral oxymel (gavaged: 0.2 mL of the syrup plus 0.8 mL of water), and SINA protocol No.1.2 (oxymel followed by sauna) groups. Treatments were given for 10 days from day 23 to 33 then sacrificed.

Significant gene expression reduction of interleukin(IL)-4, IL-5, and MUC5AC and increase of interferon(IFN)-γ and IFN-γ/IL-4 ratio and decreased perivascular and peribronchial inflammation, goblet cell hyperplasia, and subsequent mucus hypersecretion in SINA group were seen compared to the untreated group. SINA lowered IL-5 and MUC5AC gene expression levels similar to the budesonide and acted better than budesonide in increasing IFN-γ gene expression up to normal level. Compared with the asthma group, sauna alone only affected MUC5AC and IFN-γ gene expressions, and oxymel alone, only reduced IL-4 gene expression, perivascular and peribronchial inflammation, and mucus hypersecretion.

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SINA Therapy: A Novel Combination Therapy of Oxymel and Sauna in Asthmatic Mice

It seems that SINA therapy alleviates asthma via immune modulation of pro-inflammatory cytokines and improvement of pathological changes in ovalbumin-induced asthma in mice, supporting the notion of innate healing power mentioned in Persian medicine literature.

Keywords: Asthma; Persian Medicine; Thermotherapy; Traditional medicine

INTRODUCTION

Asthma is one of the most common and complicated respiratory system disorders and a major problem in public health worldwide.[1] Not only does this respiratory disease affect all aspects of a patient's life, but also has a heavy burden on families and societies.[2,3] According to World Health Organization (WHO) reports, it is estimated that around 262 million people suffered from asthma in 2019 globally.[3]

The main characteristics of asthma include increased mucus secretion, bronchial spasm, lung eosinophilia, and high serum levels of IgE.[4,5] As an essential part of the immune system, type-2 T-helper (Th2) cells generate cytokines such as interleukin (IL)-4, IL-5, and IL-13. Increased *IL-4*, *IL-5*, and *IL-13* gene expression play an important role in the development of asthma. IL-4 arouses allergic response in the lungs, induces multiplication of Th2 cells, and upregulates the synthesis of IgE which has a crucial role in bronchial hyper-responsiveness and airway inflammation. IL-5 stimulates eosinophils and their migration in bronchi, while IL-13 increases *MUC5AC* gene expression.[6-13] Moreover, the increase in *MUC5AC* gene expression leads to a change in mucosal composition, an increase in gel viscoelasticity, mucus transport impairment, and consequently airway obstruction.[14]

On the other hand, IFN-γ -a Th1 cytokine- modulates the production of Th2 cytokines and could be effective in the amelioration of asthma.[15-16] Therefore, it seems that the Th1/Th2 cytokine balance with Th1 dominancy could be a proper approach and indicator of improvement in asthma management.[17]

In recent decades, intake of medications such as inhaled corticosteroids and beta-agonists has helped improve asthma control and reduce disease severity.[18] However, low adherence to medication regimens due to concerns related to drug side effects has been reported in as many as 60-70% of asthmatic patients according to some studies.[19] Costs, side effects, and lower availability of conventional medicine in some regions have promoted the use of complementary and alternative medicines (CAMs) for the treatment of chronic diseases.[20]

The effect of alternative medicine in the treatment of asthma was previously shown.[21] One of the most famous and authentic traditional medical schools, particularly in the Middle East region is Persian Medicine (PM), which can be referred to for novel CAM prevention or treatment modalities in the above-mentioned context.[22] PM introduces several approaches for the improvement of asthma; however, sufficient evidence-based scientific research is not available for many of them yet.[23]

In PM literature, "*Rabv*" is the nearest equivalent to asthma.[23,24] In the treatment of such a condition, the importance of blood circulation and the role of the heart is noted by Persian sages.[25]

In previous articles based on PM, a series of protocols have been introduced under the general name of *"SINA therapy protocols"* [25-32] that focus on evenly distributing blood throughout the body by opening the vessels, thinning the blood, and strengthening the heart pump. Some of these protocols have already been appreciated and worked out by several faculties for a variety of diseases.[27,28,30-32] As PM literature considers asthma similar to most other chronic diseases, to be a consequence of the accumulation of waste products and cold phlegm in the lungs or related end-organs, there is a notion - in PM point of view - for potential ability of the heart and circulation to confront it leading to symptom relief or cure whenever the obstacles are removed from between the heart and the affected organs.[33-35]

The SINA protocol No.1.2 adapted for mice consists of warm oxymel gavage followed by 30 minutes of 37°C heat exposure in a closed incubator (sauna chamber). Oxymel in the abovementioned protocol is a PM beverage that is a mixture of vinegar and honey/sugar and has plenty of usage in the treatment of different conditions.[27,36,37]

Thus, the current study was designed to investigate the effects of SINA 1.2 therapy on gene expression of asthmatic factors (*IL-4*, *IL-5*, *IFN-γ*, and *MUC5AC*) and

lung pathology in ovalbumin (OVA)-induced allergic asthmatic mice.

MATERIALS AND METHODS

Forty-two male BALB/c mice (6–8-week-old weighing 15-20 g) obtained from Iran's Pasteur Institute were used for this study. Mice were kept in the following conditions: (1) pathogen-allergen-free environment; (2) unrestricted access to food and water; (3) 22–24°C temperature; (4) 45-65% humidity; and (5) circadian cycle with 12 hours of light and 12 hours of darkness.

Animals were randomly divided into six groups including a control group, a sensitized group with OVA (asthma group), and four groups sensitized and treated with budesonide as standard treatment (Budesonide group), treated with oxymel (Oxymel group), treated with sauna (Sauna group), and treated with oxymel followed by sauna (SINA group). Animal investigations were conducted based on ethical standards and after written approval by the animal ethics committee of Tehran University of Medical Sciences. (Ethics committee code No. IR.TUMS.VCR.REC.1398.221).

Induction of Asthma and Treatment

Mice were sensitized with the intraperitoneal infusion of 20 µg OVA (Sigma Chemical Co., St. Louis, USA) in 100 µL normal saline plus 50µl aluminum hydroxide (Sigma Chemical Co., St. Louis, USA), on the 1st and 14th day. Then, they were challenged with intranasal exposure to an aerosolized solution of 1% OVA produced by an ultrasonic nebulizer (Rossmax NA100, Taiwan) for 30 min per day on days 22, 24, 26, 28, 30, 32, and 34.[38] In the control group, injection and nebulization of saline were used instead of OVA (Figure 1).

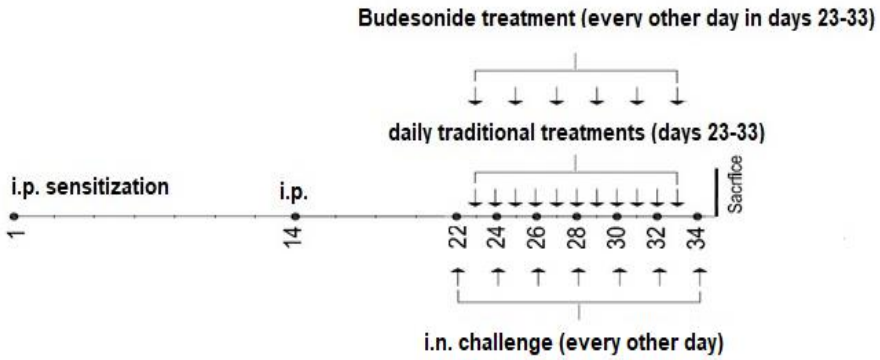

Figure 1. Timeline of asthma induction (sensitization and challenge) and treatments. i.p.: intraperitoneal, i.n.: intranasal.

During asthma induction, the treatment was administered for 10 days (Figure 1). The control and asthma groups did not receive any treatment. The oxymel group was gavaged with 0.1 mL of warm (45°C) oxymel; a dose determined according to pilot studies, prescribed dosages in PM clinics, and previous studies adjusted to the weight of mice.[27,28] The sauna group was placed in a closed warm dry incubator (sauna chamber) of 37°C for 30 minutes. The SINA group received warm oxymel gavage followed by placement in the sauna chamber. Mice in the budesonide group were treated with 1 mg nebulized budesonide (Cipla, India) by administering 200 µg/puff every 5 minutes for 25 minutes.[39]

Oxymel was prepared by heating 100 mL of vinegar (pH: 3.37) with 200 mL of water.[40] Before the solution came to a boil, 300 g sugar was added and stirred gently. After the sugar dissolved and the solution came to a boil, the heat was turned down immediately. To prepare a gavage solution, 0.2 ml of the syrup (pH: 3.52) was dissolved in 0.8 ml of water and then cooled down to 45°C after being heated again up to 100°C.[27]

Specimens Preparations for Tests

Mice were subjected to anesthesia and analgesia with an i.p. injection of a combination of 100 mg/kg ketamine (Alfasan, Netherlands) and 10 mg/kg

xylazine (Alfasan, Netherlands) solution.[41] Bronchoalveolar lavage fluid (BALF) was prepared from four mice in each group by insertion of a catheter into the trachea, and injection followed by aspiration of sterile normal saline.[42]

The left lung of the three remaining mice in each group was excised and fixed with 10% neutral buffered formalin for 48 h. Subsequently, lung tissues were embedded in paraffin after alcohol gradient treatment, and 4 μm-thick sections were prepared and stained with hematoxylin and eosin (H&E) for a general histopathology survey.

RNA Extraction and Quantitative Real-time-PCR Study

The pure total RNA was extracted from the BALF fluid's cells; using TRIzol (Invitrogen life technologies, NY, USA).

Samples of extracted RNA were reverse transcribed to produce first-strand cDNA; using a cDNA synthesis kit (Maxima First Strand cDNA Synthesis Kit, Thermo Scientific, Rockford, IL, USA), which contains a double-strand specific DNase (dsDNase) that specifically eradicates polluting genomic DNA from RNA samples.

Quantitative real-time PCR to measure mRNA expression was performed and analyzed using a Rotor-Gene SYBR Green PCR Kit and a Rotor-Gene Q thermal cycler (Qiagen, Hilden, Germany). The primer sequences for the four target genes (*IL-4*, *IL-5*, *MUC5AC*, and *IFN-γ*) and one primer pair for *glyceraldehyde-3-phosphate dehydrogenase (GAPDH*) as a reference gene (endogenous control) are shown in Table S1. The latter was obtained from previous studies with BLAST search being performed using the BLAST and IDT Oligo-Analyzer (version 3.1) to confirm their specificity.[43]

Histopathology Study

Under light microscopy, eosinophil infiltration around vessels and bronchi (perivascular and peribronchial inflammation), goblet cell hyperplasia, and mucus hypersecretion were scored based on a previous study.[43] Briefly, the perivascular and peribronchial inflammation was scored by counting the layers of inflammatory cells formed around the vessels and airways. Goblet cell hyperplasia was scored by calculation of Goblet Cell Index (GCI), while mucus hypersecretion was rated based on the average percentage of the mucus-positive area in randomly selected bronchi.[44,45]

Histopathologic evaluation was performed individually by three histopathology experts. Photomicrographs were taken with an Olympus B × 50 microscopes (Olympus, Japan) equipped with a Leica DFC 320 digital Camera (Leica, Germany).

Statistical Analysis

Data for gene expression are expressed as mean ± standard deviation of the mean (SD) and statistical comparisons of the data among various groups were performed using the Brown-Forsythe and Welch's ANOVA test followed by Dunnett's T3 multiple comparisons test. The *IFN-γ/IL-4* gene expression ratio comparisons were accomplished by ordinary one-way ANOVA followed by Tukey's multiple comparisons test.

The Data for pathological results are expressed as median followed by the inter-quartile range or IQR and comparisons among groups were performed using the non-parametric two-tailed Mann-Whitney U statistic test.

All Statistical analyses were performed using GraphPad Prism 8 (GraphPad Software, USA). A P value of lower than 0.05 was considered to indicate a statistically significant difference for all mentioned analyses.

RESULTS

***IL-4, IL-5, MUC5AC,* and *INF-γ* Relative Gene Expression**

In the asthma group, mRNA expressions of IL-4, IL-5 and MUC5AC were significantly higher ($p<0.001$ for IL-4 & MUC5AC and $p<0.01$ for IL-5) than in the control group (Figures 2-4), while that of INF-γ was significantly lower ($p<0.001$, Figure 5).

In the budesonide group, gene expression of *IL-4*, *IL-5*, and *MUC5AC* significantly declined ($p<0.01$ for IL-4 and MUC5AC and $p<0.05$ for IL-5), while that of INF-γ significantly improved compared to the asthma group ($p<0.001$), (Figures 2-5). Additionally, gene expression of *IL-5* in the budesonide group did not differ significantly from the control group. Despite the improvement of all gene expressions in the budesonide group, levels of IL-4, MUC5AC, and INF-γ remained significantly different from the control group ($p<0.001$ for all), (Figures 2-5).

In the sauna group, gene expression of merely *MUC5AC* was significantly reduced (p<0.01), while *INF-γ* was significantly increased (p<0.05) compared to the asthma group. However, gene expressions of *MUC5AC* and *INF-γ* did not reach levels of the control, budesonide, or SINA groups (p<0.001 for all), (Figures 2-5).

In the Oxymel group, gene expression of only *IL-4* was significantly reduced (p<0.01) compared to the asthma group, yet it did not reach the levels of control, budesonide, or SINA groups (p<0.001 for all), (Figures 2-5).

In the combined treatment group (Oxymel+Sauna) named the SINA group, gene expressions of *IL-4*, *IL-5* and *MUC5AC* were significantly declined (p<0.05 for IL-5 and p<0.001 for IL-4 and MUC5AC), and gene expression of *INF- γ* was significantly elevated compared to the asthma group (p<0.01) (Figures 2-5).

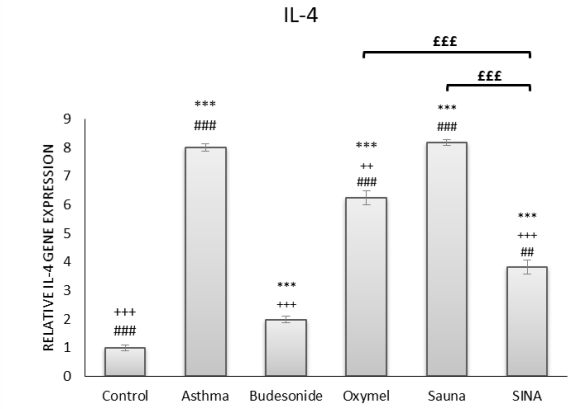

Figure 2. Values of *IL-4* gene expression in control, untreated asthma, budesonide, oxymel, sauna, and SINA groups. The significant difference compared to the control group: ***p<0.001. Significant difference compared with untreated asthma group: ++ p<0.01, +++ p<0.001. Significant difference compared with budesonide group: ## p<0.01, ### p<0.001. The significant difference between the oxymel and sauna groups compared to the SINA group: £ £ £ p<0.001. Statistical comparisons were made using Brown-Forsythe & Welch's ANOVA test followed by Dunnett's T3 multiple comparisons test.

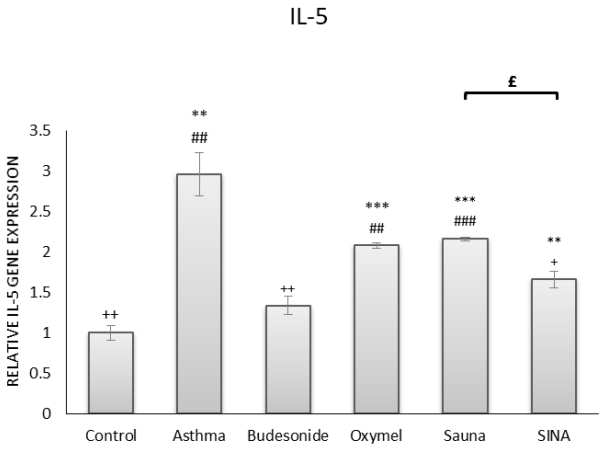

Figure 3. Values of *IL-5* gene expression in control, untreated asthma, budesonide, oxymel, sauna, and SINA groups. Significant difference compared with control group: ** p<0.01, *** p<0.001. Significant difference compared to untreated asthma group: + p<0.05, ++ p<0.01. Significant difference compared with budesonide group: ## p<0.01, ### p<0.001. The significant difference between oxymel and sauna groups compared with the SINA group: £ p<0.05. Statistical comparisons were made using Brown-Forsythe & Welch's ANOVA test followed by Dunnett's T3 multiple comparisons test.

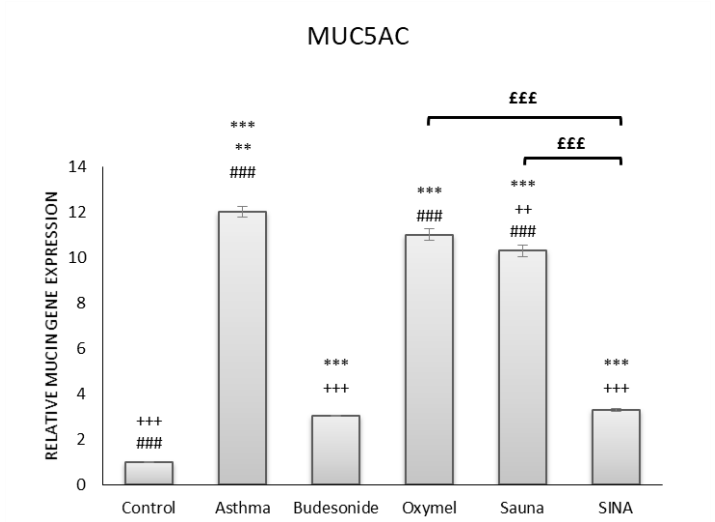

Figure 4. Values of *MUC5AC* gene expression in control, untreated asthma, budesonide, oxymel, sauna, and SINA groups. The significant difference compared with the control group: ***p<0.001. Significant difference compared with untreated asthmatic group: ++ p<0.01, +++ p<0.001. The significant difference compared with the budesonide group: ### p<0.001. The significant difference between oxymel and sauna groups compared with the SINA group: £££ p<0.001. Statistical comparisons were made using Brown-Forsythe & Welch's ANOVA test followed by Dunnett's T3 multiple comparisons test.

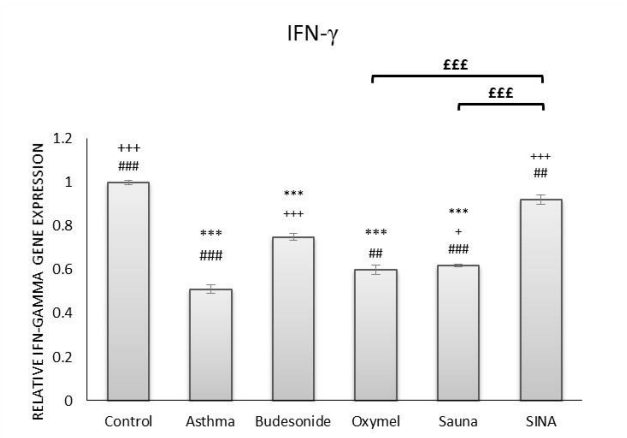

Figure 5. Values of *IFN-γ* gene expression in control, untreated asthma, budesonide, oxymel, sauna, and SINA groups. The significant difference compared with the control group: ***p<0.001. Significant difference compared with untreated asthma group: + p<0.05, +++ p<0.001. Significant difference compared with budesonide group: ## p<0.01, ### p<0.001. The significant difference between oxymel and sauna groups compared with the SINA group: £££ p<0.001. Statistical comparisons were made using Brown-Forsythe & Welch's ANOVA test followed by Dunnett's T3 multiple comparisons test.

Also, gene expressions of *IL-5* and *MUC5AC* in the SINA group were similar to the budesonide group, but that of *INF-γ* increased remarkably higher than the budesonide group (p<0.01) and reached the levels of the control group.

The SINA group showed a significantly higher effect compared to the Oxymel and Sauna groups in all gene expressions except similar reduction in *IL-5* with oxymel (p<0.001 for all except p<0.05 for SINA vs. sauna, Figures 2-5).

IFN-γ/IL-4 Gene Expression Ratio

The *IFN-γ/IL-4* gene expression ratio was calculated as an index of Th1/Th2 balance. This ratio decreased significantly in the untreated asthma group compared with the control group (p<0.001). Although treatment of sensitized animals with budesonide led to a significant increase in Th1/Th2 balance compared with the asthma group (p<0.001), it kept a significant distance from that of the control group (p<0.001), (Figure 6).

The ratio of *IFN-γ/IL-4* gene expression in oxymel and sauna treatments alone did not differ significantly from the asthma group.

The *IFN-γ/IL-4* gene expression ratio in the SINA group improved significantly compared with all other groups (p<0.001 for all).

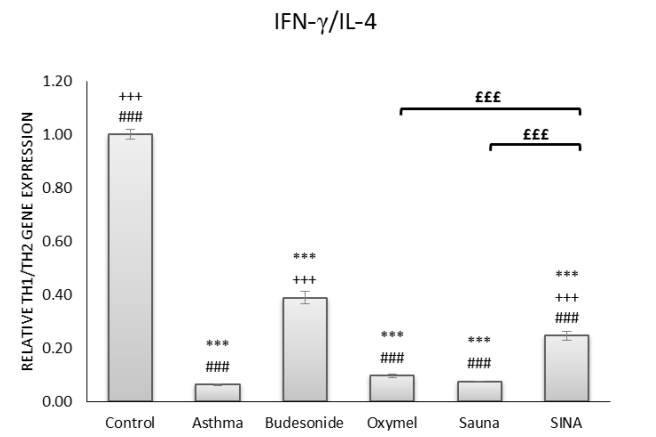

Figure 6. Values of Th1/Th2 balance (ratio of *IFN-γ* to *IL-4* gene expression) in control, untreated asthma, budesonide, oxymel, sauna, and SINA groups. The significant difference compared to the control group: ***p<0.001. The significant difference between all groups compared with the untreated asthma group: +++ p<0.001. The significant difference between all groups compared with the budesonide group: ### p<0.001. The significant difference between oxymel and sauna group compared with SINA group: £££ p<0.001. The statistical comparisons were made; using an ordinary one-way ANOVA test followed by Tukey's multiple comparisons test.

Histopathological Findings

Data for pathological results are expressed as median followed by their interquartile range or IQR in Table 1. Histopathological study of tissue sections (Figure 7 and Table 1) demonstrated pathological changes of asthma including perivascular and peribronchial infiltration of inflammatory cells besides goblet cell hyperplasia and mucus hypersecretion compared with the control group (p<0.05 for all 4 indices).

Treatment with budesonide significantly reduced all pathologic indices compared with the asthma group (p<0.05 for all 4 indices) but did not show a significant difference compared with the control group.

The sauna group was not significantly different from the asthma group in terms of pathologic indices, but all indices remained significantly higher than the control, budesonide, and SINA groups (p<0.05 for all).

In the oxymel group, peribronchial, perivascular inflammation, and mucus secretion were significantly reduced compared with the asthma group (P<0.05 for all), yet all four indices remained significantly higher than the control group (p<0.05 for all 4 indices) and were only similar to budesonide and SINA groups in mucus hypersecretion.

The mixed intervention, namely SINA protocol reduced all pathologic indices similar to the budesonide and control groups which were significantly lower than the asthma group (p<0.05 for all four indices).

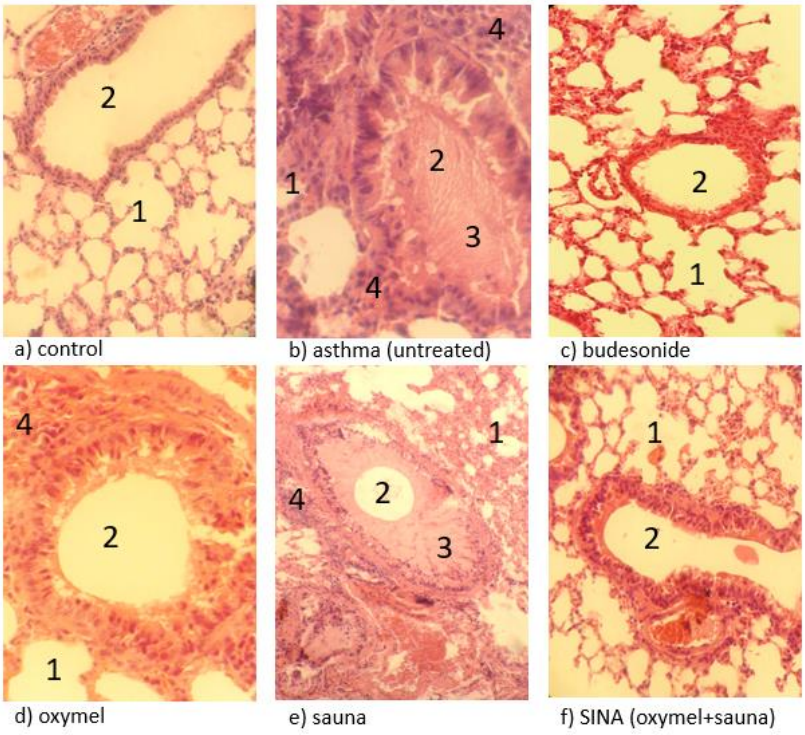

Figure 7. Histopathological analysis of lung tissues stained with Hematoxylin-eosin displaying alveoli (1), bronchioles (2), mucus plug (3), and infiltration (4): (a) The control group treated with saline showing no infiltration and open alveoli and bronchioles; (b) The untreated asthma group sensitized and challenged by ovalbumin showing bronchial lumen obstruction with mucus plug and massive infiltration with inflammatory cells and alveolar collapse; (c) The asthma group treated with budesonide showing airway clearance from mucus and reopening of alveoli; (d) The asthma group treated with oxymel alone showing a reduction in infiltration and mucus accumulation; (e) The asthma group treated with sauna alone showing slight changes in infiltration and mucus accumulation; (f) The asthma group treated with SINA showing marked reduction in infiltration and mucus accumulation and reopening of alveoli and bronchioles.

Table 1. Histological analysis of lung tissue: Histological results in study groups

Groups	Perivascular inflammation	Peribronchial inflammation	Goblet cell hyperplasia	Mucus hypersecretion
Control	1.15 (0.18)$^{+}$	1.2 (0.30)$^{+}$	1 (0.15)$^{+}$	5 (3.75)$^{+}$
Asthma	3.80 (0.30)$^{*\ \#}$	3.85 (0.18)$^{*\ \#}$	3.25 (0.73)$^{*\ \#}$	75 (18.75)$^{*\ \#}$
Budesonide	1.20 (0.23)$^{+}$	1.35 (0.30)$^{+}$	1.2 (0.38)$^{+}$	17.5 (18.75)$^{+}$
Oxymel	2.50 (0.60)$^{*\ +\ \#\ £}$	2.35 (0.68)$^{*\ +\ \#\ £}$	2.65 (0.45)$^{*\ \#\ £}$	50 (18.75)$^{*\ +}$
Sauna	3.35 (0.53)$^{*\ \#\ £}$	3.35 (0.68)$^{*\ \#\ £}$	3.1 (0.43)$^{*\ \#\ £}$	62.5 (25.00)$^{*\ \#\ £}$
SINA	1.50 (0.23)$^{+}$	1.5 (0.45)$^{+}$	1.5 (0.23)$^{+}$	25 (11.25)$^{+}$

Values are presented as median (Interquartile range or IQR). Statistical significance for the difference between the data of asthma and control groups: * p<0.05. Statistical significance compared with asthma group: + p<0.05. Statistical significance compared with budesonide group: # p<0.05. The significant difference between oxymel and sauna groups compared with the SINA group: £ p <0.05. Statistical comparisons were made; using a two-tailed nonparametric Mann-Whitney U test.

DISCUSSION

In the present study, immunological and pathological changes in the asthma group, were similar to the results of previous studies using a similar method of sensitization,[46-50] confirming the sensitization and induction of an animal model of asthma in rodents.

Standard treatment with inhaled budesonide used in this study, like previous studies,[46,51] effectively reduced gene expression levels of *IL-4*, *IL-5*, and *MUC5AC* as well as histopathological indices and significantly increased expression of *IFN-γ* gene compared with the untreated asthma group. Budesonide recovered *IL-5* gene expression level and all pathological indices close to the indices of the control group. Similar to previous studies,[46,52,53] budesonide also caused a significant rise in the *IFN-γ/IL-4* gene expression ratio. All these findings justified the selection of budesonide treatment as a control treatment in the animal model of asthma used in this study.

Sauna therapy alone significantly reduced expression of *MUC5AC* and increased *INF-γ* gene expression compared with the asthma group, which revealed some therapeutic effects for this modality. However, it was unable to induce a significant difference in any pathologic indices compared with the untreated asthma group and maintained significant differences in all gene expressions and histopathologic indices compared with the control, budesonide, and SINA groups. This revealed that despite therapeutic effects, the sauna alone is not an effective asthma treatment. Sauna has shown effects on various conditions such as cardiovascular, skin, rheumatoid, and lung diseases.[54] A long-term prospective cohort study by Kunutsor and colleagues indicated a significant inverse association between frequent sauna bathing and risk of acute and chronic respiratory conditions.[55] Furthermore, a six-week sauna treatment program for Thai patients with allergic rhinitis enhanced peak nasal inspiratory flow and lung functions.[56] Despite such findings, to the best of our knowledge, no studies have been conducted on the therapeutic effect of simple sauna therapy on immune modulation or pathologic indices in asthma.

In our study, oxymel treatment alone significantly reduced gene expression of *IL-4* and mucus hypersecretion similar to budesonide. Despite these findings, oxymel did not significantly change any other studied gene expression, *IFN-γ/IL-4* gene expression ratio, or pathologic indices and showed significantly lower effects in all measured genetic and histopathologic indices compared with the control and budesonide groups. Therefore, having small therapeutic effects, oxymel alone could not be regarded as an effective treatment for asthma. There is also no data to show the effect of different types of simple oxymel formulations including honey or sugar made types on immune modulation or pathologic indices in asthma, despite many historical and traditional claims about its effect on respiratory diseases such as asthma.[57,58] However, the significant effect of oxymel on "quality of life" scores (SGRQ scores) including the SGRQ symptom score, activity score, and total score in asthmatic patients has been shown previously,[59] which are consistent with the results of the current study. In the above-mentioned study, this positive effect was attributed to the temperament-modifying activity of simple honey oxymel in the respiratory system. Although a simple "sugar-made" oxymel used in our study also showed slight therapeutic effects probably due to a less warm temperament of sugar compared to honey- according to PM principles - surprisingly it showed augmented effects not seen from sauna nor oxymel alone when given before sauna in the SINA protocol.

Finally, consecutive administration of oxymel and sauna as SINA (No.1.2) protocol, significantly diminished *IL-4*, *IL-5*, and *MUC5AC* gene expressions and all histopathologic scores and boosted *IFN-γ* gene expression and *IFN-γ/IL-4* gene expression ratio. SINA not only lowered *IL-5* and *MUC5AC* gene expression levels statistically similar to the budesonide but also acted even more effectively than budesonide in increasing *IFN-γ* gene expression levels up to normal control levels.

Treatment with SINA, unlike sauna alone, was able to significantly change gene expression levels of *IL-4*, *IL-5*, and *INF-γ/IL-4* ratio compared to the untreated asthma group. On the other hand, although sauna was also able to significantly affect *MUC5AC* and *INF-γ* gene expressions compared with the asthma group, this change was not as much as SINA. Considering the above and also the significant difference between SINA and sauna groups in all pathologic scores, it seems that the combination of oxymel and subsequent sauna as SINA protocol acts different and more prominently

than sauna alone in lowering the inflammatory process in induced asthma in BALB/c mice.

Treatment with SINA compared to oxymel alone also reduced gene expression levels of *IL-5*, and *MUC5AC* and increased the *INF-γ* and *INF-γ/IL-4* ratio significantly higher than oxymel alone. Although oxymel also reduced *IL-4* gene expression significantly compared with the asthma group, this reduction was not as much as that induced by SINA. Regarding pathologic indices, oxymel reduced perivascular and peribronchial inflammation and mucus hypersecretion compared with the untreated asthma group, but this reduction was lower than SINA treatment except for mucus reduction. Therefore, it seems that the combination of oxymel and subsequent sauna as SINA protocol is more promising than oxymel alone in lowering the inflammatory process in induced asthma in BALB/c mice.

These results showed similarly and even in some indices higher efficacy of SINA protocol compared with inhaled budesonide in attenuating various aspects of asthma, suggesting potential novel and safer modalities such as SINA in asthma management.[60,61]

As mentioned previously, PM resources mention a variety of herbal and non-herbal approaches for the treatment of asthma and allergies.[11,13] Although many studies have shown promising anti-asthmatic effects for some PM-recommended herbs including *Nigella Sativa*, *Ocimum basilicum*, *Urtica dioica*, *L Lavendula*, *Curcuma longa*, *Silybium marinum,* etc.[15,17] no research was found to study non-herbal PM treatments like SINA. In this study, a combination of two treatment modalities - oxymel followed by sauna- with a novel and unique PM rational hypothesis to work on the heart and circulation, was studied and proven effective. Results support the notion that the body may heal itself by its innate power, especially when aided and supported generally without necessarily consuming drugs.[62]

In the present study expression of four genes, *IFN-γ/IL-4* gene expression ratio as an index of Th1/Th2 balance and pathological changes in the lungs were examined demonstrating the effect of SINA protocol in an animal model of asthma. Further studies on the effect of the SINA protocol including assessment of total and differential WBC and oxidative markers both in the blood and the BALF are yet needed to complete the picture. In addition, clinical studies are needed to assess the effect of the SINA protocol on asthmatic patients.

This study demonstrated that SINA protocol No.1.2 decreases expressions of TH2 cytokines while increasing that of TH1 cytokines, shifting TH1/TH2 balance in favor of cell-mediated immune (CMI) response, and reducing lung pathologic indices in a mouse model of allergic asthma for the first time, indicating promising potential therapeutic effects of SINA No.1.2 in diverse pathologic conditions where CMI dominancy is preferred (e.g., asthma and allergic reactions, viral and non-viral intracellular infections and cancer).

By emphasizing the effectiveness of a novel protocol deduced from Persian Medicine principles – SINA therapy - these results support the notion of the self-healing ability of the homeostatic innate power claimed by PM literature.

However, more experiments are needed to elucidate the detailed mechanisms of SINA protocol on airway inflammation in animal models of asthma, and further clinical investigations are needed to confirm its outcome in asthmatic patients.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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