Original Article

OPEN ACCESS

Therapeutic Effect of Avishan-e-Shirazi (*Zataria Multiflora* Boiss) Root Extract on Oxidative Stress Markers in Moderate Asthmatic Patients

Farah Khanum[1], Noor Fatima[1], Noor a Fatima[2], Sheeza Munawar[1], Muntaha Jaffar[1], Atiqa Hassan[1], Alina Akbar[3] and Misbah Arshad[1*]

[1]University Institute of Diet and Nutrition Sciences, The University of Lahore, Lahore, Pakistan
[2]University of South Asia, Lahore, Pakistan
[3]Institute of Southern Punjab, Multan, Pakistan

ARTICLE INFO

*Corresponding Author:

Misbah Arshad
University Institute of Diet and Nutrition Sciences, The University of Lahore, Lahore, Pakistan
fatimamisbah10@gmail.com

ABSTRACT

Avishan-e-Shirazi (*Zataria Multiflora Boiss*) is a plant widely recognised for its medicinal and culinary purposes. **Objectives:** To evaluate the therapeutic effect of plant's root extract on pulmonary function tests and oxidative stress markers among moderate Asthma tic patients. **Methods:** A total participant of 66 patients with pulmonary disease, 30-40 age, were selected for the 2-month research trial. Individuals were enrolled and distributed equally in two groups, 33 each. In the control group, 53.8% of individual were man, and 46.2% were women. In the treatment group, 49.1% of participants were man, and 50.9% were women. The groups G0 was considered as a control group with no root extract, and group G1 where conventional treatment and root extract were advised to participants. The participants were selected from the Jinnah Hospital, Pakistan. The 5mg/kg/day dried root extract supplements 3 times a day were given to the individuals daily for 2 months. **Results:** The mean age group of Asthma tic patients enrolled in the study was 35 ± 2.98 years in G0, and in G1, it was 34 ± 3.05. The mean BMI was 31.34 and 30.98 kgm-2 in both groups, respectively. There was a significant improvement in FEV1 levels and MDA enzyme levels with a p-value less than 0.05. The NO2 levels were also better. Similarly, the enzymes thiolase and SOD levels also improved with a p-value less than 0.05 in the treatment group. **Conclusions:** The current study concluded that Avishan-e-Shirazi root extract could improve the pulmonary function and inflammation among Asthma patients.

INTRODUCTION

Sudden episodes of breath shortening characterise bronchial Asthma. The heaviness is felt around the chest area, and wheezing is caused by temporary constriction of the bronchial airways. This constriction is caused by muscle spasms, swelling of the mucosal lining, and an increase in sticky bronchial secretions, which result from an inflammation reaction in the bronchial walls [1]. Asthma is a common respiratory condition affecting millions of people globally. The current estimate is that around 334 million individuals live with Asthma; according to the latest record, the number of individuals with Asthma will be one hundred million. It will be the approximation for the year 2025. In Pakistan, the incidence of Asthma varies significantly between different regions, with reports showing a range from 4.3% to 31.58% [2]. Individuals with severe Asthma are at higher risk of experiencing a lower quality of life, permanent airway blockage, hospitalization, and even death. To lower the impact of the disease, biologics may be necessary [3]. Asthma symptoms can change over time and become more severe, leading to respiratory failure during episodes of exacerbation [4]. Asthma symptoms can be triggered by various factors,

including infections, allergens such as pollens and molds, exercise, certain drugs can also trigger, and these drugs are anti-inflammatory and non-steroidal mostly, smoking, exposure to fumes from chemicals or herbs, emotional stress, contact with household pets, dust, and mites [5]. This disease has four stages, ranging from mild to moderate and intermittent to high-risk levels [6]. Asthma can present with symptoms such as wheezing, breathing difficulty, coughing, and heaviness around the chest area. All these symptoms are due to one main reason: the blockage of microtubules of the lung's airways [7]. Asthma is a frequent health issue in Pakistan that has become more prevalent lately. Research in the Journal of Pakistan Medical Association estimates that 5-7% of Pakistan's population suffers from Asthma. The research also discovered that children are more likely than adults to develop Asthma, with an estimated frequency of 9.8% in children ages 13 to 14 [8]. The most common cause of sudden Asthma exacerbations in children and adults, particularly children, is viral infections of the respiratory system. Up to 75% of wheezing episodes in infants and 50-70% in adults may be caused by viruses [9]. Most cellular intrudes include neutrophils, lymphocytes, eosinophils, mast cells, macrophages and basophils. Asthma heterogeneity is shown by the vast variations in these cell ratios across individuals [10]. Avishan-e-Shirazi is a plant widely recognised for its medicinal and culinary purposes and it is mainly grown in countries like Iran, Pakistan and Afghanistan [11]. Conventional medicine has been using the aqueous extract of Avishan-e-Shirazi for various therapeutic purposes, primarily for treating coughs, bronchitis, and other respiratory tract disorders. This native herb also caters the oral hygiene due to its bactericidal properties. Carvacrol, thymol, linalool, and p-cymene are the active compounds present in Avishan-e-Shirazi [12]. Due to their diverse pharmacological properties, natural products and their components are an essential source of potential new drugs. Avishan-e-Shirazi, for example, has been shown to have antimicrobial effects. Its nutrition composition showed that it contains 101 kcal, carbohydrates 24.45g, protein 5.56g, fat 1.68g, fiber 14g, vitamin A 1010 IU, vitamin C 160mg, vitamin K 1714.5μg, calcium 405mg, iron 17.45mg, magnesium 160mg, potassium 609 mg [11]. The use of Avishan-e-Shirazi in traditional medicine has been documented for centuries, and its pharmacological properties have been extensively studied [12]. In recent years, the plant has gained increased recognition for its medicinal properties. Various diseases can be targeted for treatment by this herb as it has anti-fungal and anti-inflammatory characteristics [13]. Avishan-e-Shirazi needs more inquiry through research as many of the valuable effects of this herb are unknown to science. The scientific knowledge is also insufficient on the mode of activity of plants. It is also important to note that Avishan-e-Shirazi should not be used as a substitute for conventional medical treatments, and individuals should always consult with their healthcare providers before using herbal remedies [14]. The use of Avishan-e-Shirazi in conjunction with other medications may also result in adverse effects, so it is essential to monitor its use and evaluate its potential benefits and risks carefully. These compounds have been shown to have relaxant effects on tracheal smooth muscle and to play a role in managing respiratory tract disorders [15]. The therapeutic effects of Avishan-e-Shirazi are likely due to its ability to modulate different physiological processes and its various pharmacological properties [16].

In this study, we focused on the therapeutic effect of Avishan-e-Shirazi root extract on the moderate asthmatic patients, who experienced symptoms and had nighttime awakenings more than once a week.

METHODS

We conducted randomized control trial (RCT) at Jinnah Hospital with the target population of patients with Asthma aged 30 to 40 years. The sample size was estimated using the method of dependent means, with pre and post treatment mean MDA values of -38.40 and 18.91, respectively, resulting in a calculated sample size of 33 in each group. Sixty-six individuals satisfied the criteria for our study and were distributed into two groups. Based on the sample population distribution, patients were assigned to either the treatment (n=33) or control (n=33) groups. The sample size was determined using the following formula:

$$t = \frac{\sum d}{\sqrt{\frac{n(\sum d^2) - (\sum d)^2}{n-1}}}$$

where d: difference per paired value
n: number of samples

Patient baseline characteristics, including anthropometrics and demographics measurements, were taken along with FEV1, oxidative stress, MDA, superoxide dismutase, thiol, nitrite and BMI. The parts of Avishan-e-Shirazi were separated, washed and air-dried. Furthermore, it was milled and extracted by percolation method performing using 1000 ml of ethanol 70% at room temperature for 72 hrs. After filtration, ethanol was evaporated at 40°C in a rotary. After that, solvent evaporation was performed by vacuum desiccator for 24 hrs, and the dried extract was stored at -20c the efficiency of this method was 16.5% [17]. The Avishan-e-Shirazi root extract provided to patients was prepared in the lab following a standard procedure to ensure uniformity in extract intervention as shown in table 1.

Table 1: Treatment Details

Variables	G$_0$ Control Group (No Treatment)	G$_1$ Treatment Group (Avishan-e-Shirazi Root Extract)
Dosage	-	5mg/ kg Avishan-e-Shirazi Root Extract
Duration	8 Weeks	8 Weeks
Frequency	-	3 Times a Day
Target Population	30-40 Years	30-40 Years

Participants were enrolled in the study if they met the inclusion criteria. Blood samples and spirometry levels (which gauge the rate of airflow) were taken from Asthmatic patients and healthy volunteers. The plasma layer was separated by centrifugation and measured oxidative stress markers was collected by the biology of stress tolerance lab, IMBB lab assistant, The University of Lahore, Lahore. All participants gave written informed consent (attached), and the information and data obtained were absolutely confidential. Participants remained anonymous throughout the experiment and were informed that they might withdraw at any time during the procedure. The experiment lasted around nine months.

RESULTS

The mean age of G$_0$ (control group) individuals was 35 ± 2.98, and that of G$_1$ (treatment group) participants was 34 ± 3.05. Individuals mean weight in G$_0$ was 78 ± 3.98, and in G$_1$ was 75.98 ± 2.67. The maximum weight observed in G$_0$ and G1 were 92 and 95 kg respectively. The study had a total of 66 participants enrolled and distributed equally in two groups with the control group, 53.8% of participants were men,

and 46.2% were women. In the treatment group, 49.1% of participants were men, and 50.9% were women respectively. The mean BMI of individual in groups G$_0$ and G$_1$ was 31.34 ± 1.56 kg/m2 and 30.98 ± 1.90 kg/m2 respectively. The maximum BMI of individuals observed in groups G$_o$, and G$_1$ was 36.4 and 37.6 kg/m2, respectively. The mean FEV1 of patients in G$_0$ was 37 ± 1.9 as compared to 41 ± 2.1 among patients in G$_1$ before treatment, with the mean MDA of patients in G$_0$ being 2.70 ± 0.1 as compared to 2.40 ± 0.1 among patients in G before the treatment (table 2).

Table 2: Characteristics of Asthma Patients Before Intervention

Variables	Control Group Mean ± SD	Treatment Group Mean ± SD
Number of Patients (N)	33	33
Age	35 ± 2.98	34 ± 3.05
Height (cm)	159.45 ± 3.34	163.45 ± 4.19
Weight (kg)	78 ± 3.98	75.98 ± 2.67
Gender (N)	M=18, F=15	M=16, F=17
BMI (Kgm-2)	31.34 ± 1.56	30.98 ± 1.90
(FEV1 %)	37 ± 1.9	41 ± 2.14
MDA (μmol/L)	2.70 ± 0.15	2.40 ± 0.1
Nitrogen Dioxide (NO2)(ppb)	6.50 ± 0.33	5.90 ± 0.30
Thiol	0.45 ± 0.02	0.50 ± 0.03
Superoxide Dismutase (SOD)(U/ml)	154 ± 1.7	157 ± 1.85

The change was noticed in the parameters of individuals post intervention of Avishan-e-Shirazi root extract 5gm daily. Improvement was seen in FEV1 oxidative stress which is explained in table 3.

Table 3: Characteristics of Individuals Pre and Post-Avishan-e-Shirazi Root Extract Treatment

Variables	Control Group Mean ± SD			Treatment Group Mean ± SD			p-value
	Pre-Intervention	Post Intervention	% Change	Pre-Intervention	Post Intervention	% Change	
(FEV1 %)	37.25 ± 1.9	39.2 ± 2.81	5.26%	41.18 ± 2.13	46.5 ± 4.30	11.4%	0.30
MDA (μmol/L)	2.70 ± 0.14	2.45 ± 0.12	16%	2.49 ± 0.19	1.80 ± 0.12	28.5	0.07
Nitrogen Dioxide (No2)(ppb)	6.50 ± 0.33	6.20 ± 0.31	4.72%	5.90 ± 0.34	4.60 ± 0.23	24.7%	0.04
Thiol	0.45 ± 0.02	0.49 ± 0.02	8.5%	0.50 ± 0.03	0.58 ± 0.05	14.8%	0.00
Superoxide Dismutase (SOD)(U/ml)	154.52 ± 1.73	161.70 ± 2.05	4.4%	157.18 ± 1.85	169.16 ± 2.45	7.36%	0.007
TNF- α (Pg/ml)	16.58 ± 1.33	17.80 ± 2.76	-	18.41 ± 1.61	15.15 ± 2.14	-	0.00
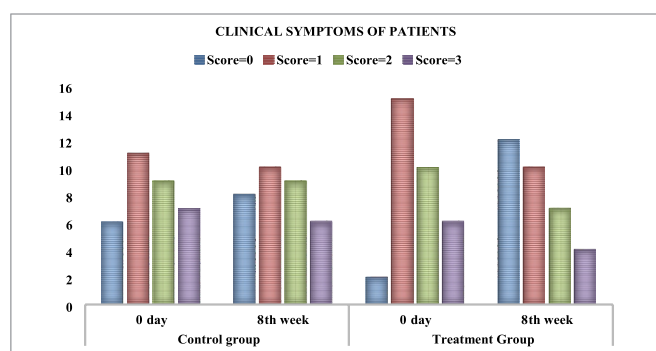
Figure 1: Clinical Symptoms of Patients in a Different Groups

Score 0= no wheezing and cough, Score 1= good sleep with slight wheezing and cough, Score2= wake up once a night, score 3= wake up more than once at night

DISCUSSION

Avishan-e-Shirazi (*Zataria multiflora*) and its components have been shown to have spasmolytic and anti-tussive characteristics that can be used to treat respiratory tract problems. A clinical trial has demonstrated the Avishan-e-Shirazi efficacy in treating acute cough both on its own and in combination with other plants significantly improved [18]. A study conducted in Iran evaluated that *Z. multiflora* is

widely used in Iranian traditional medicine for the treatment of cough, chest problems, oral cavity infection, dyspepsia, and other problems of the respiratory system [19]. In our study, the mean FEV1 of patients in G_0 was 37 ± 1.9 as compared to 41 ± 2.1 among individuals in G_1 pre the treatment, whereas the mean FEV1 of group G_0 and G_1 post treatment of Avishan-e-Shirazi was 42 ± 2.8 and 46 ± 4.3 respectively. A study conducted to determine the effect of *Z. multiflora* on wheezing and FEV1 along with plasma levels of nitrite included 40 Asthmatic patients who were randomly divided to determine the dosage effect of *Z. multiflora* on respiratory health. they were given different dosages of multiflora in a double-blind manner to increase the efficiency and durability of the test. Forced expiratory volume in 1 second, NO_2 and wheezing during exercise bout or throughout the day were measured at the advent of treatment, which was the baseline measurement (pre-treatment), one month after the treatment and at the end of treatment was recorded to assess the efficacy of the treatment provided in different dosages. After the study, it was elucidated that FEV1 % was significantly improved in the study participants with the intervention of *Z. multiflora* as compared to a control group with p-values ranging from < 0.01 to <0.001, indicating that the results were statistically significant. FEV1 was 7.9 ± 7.16 in the treatment group of low *multiflora* dosage, which is 5.0mg/ day at baseline, which improved to 8.91 ± 6.36 after treatment which showed a difference of 1.01 ± 3.17 indicating improvement in FEV1 with the p-value was <0.05 showing significant results. The second group with a 10mg/day dosage of multiflora had FEV1 9.84 ± 19.68 in the advent which altered to 12.28 ± 9.88 after the intervention. It showed an improvement of 3.72 ± 11.7 with p-value <0.001 among the study participants, indicating the potential beneficial impact of the root as all the other factors were kept constant in the study [20]. The mean NO_2 of patients in G_0 was 6.50 ± 0.33 as compared to 5.90 ± 0.30 among individuals in G_1 pre the treatment, whereas the mean NO_2 of group G_0 and G_1 post treatment was 6.20 ± 0.31 and 4.6 ± 0.23 respectively. A study conducted to determine the effect of *Z. multiflora* on wheezing and FEV1, along with plasma levels of nitrite, included 40 Asthmatic patients. The mean age of participants was 46.6 ± 1.23 years, with Asthma severity of 4.5 ± 3.06. The participant was randomly divided into four groups in a double-blind manner to validate the study results. The aim was to determine the dosage base and overall effect of *Z. multiflora* on respiratory health. After the study, it was concluded that NO2 was significantly improved in the study participants with the intervention of *Z. multiflora* compared to a control group with p value ranging from < 0.01 to <0.001, indicating that the results were statistically significant. NO_2 was -4.33 ± 37.79 in the treatment group of low multiflora dosage, which is 5.0mg/ day at baseline, which decreased to -5.59 ± 37.03 after treatment, which showed the difference of 1.33 ± 11.59 indicating improvement in NO_2 with the p-value was <0.05 showing significant results. The second group with 10mg/ day dosage of *Z. multiflora* had -4.23 ± 8.91 in the advent, which altered to -16.77 ± 12.85 after the intervention. it showed an improvement of -13.15 ± 9.61 with p-value <0.01 among the study participants, indicating the potential beneficial impact of the root as all the other factors were kept constant in the study [20].

CONCLUSIONS

The current study concluded that Avishan-e-Shirazi root extract effectively improves Asthma symptoms among patients. This study manifested significant decrease in inflammatory markers. There was no significant total difference in BMI and weight changes between study participants. Avishan-e-Sherazi root extract showed emerging results with natural treatment. However, the mechanism of action of this extract in Asthma symptoms is still unknown. Further research is needed to understand its therapeutic mechanisms and to develop new treatments based on its extracts.

Authors Contribution

Conceptualization: MA
Methodology: MI
Formal analysis: SM
Writing-review and editing: FK, NF, NAF, AH, AA

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

REFERENCES

[1] Danish MJ. Medical Diagnosis and Management. 12th edition. Karachi, Pakistan: Paramount Books (Pvt.) Ltd.; 2020.

[2] Sabar MF, Akram M, Awan FI, Ghani MU, Shahid M, Iqbal Z *et al*. Awareness of Asthma Genetics in Pakistan: A review with some recommendations. Advancements in Life Sciences. 2018 Nov; 6(1): 1-0.

[3] Brusselle GG and Koppelman GH. Biologic therapies for severe asthma. New England Journal of Medicine. 2022 Jan; 386(2): 157-71. doi: 10.1056/NEJMra2032 506.

[4] Lambrecht BN, Hammad H, Fahy JV. The Cytokines of Asthma. Immunity. 2019 Apr; 50(4): 975-91. doi: 10.1016/j.immuni.2019.03.018.

[5] Mustafa M, Mustafa R, Asif R, Mazhar M, Munir N, Mahmood Z. Pharmaceutical monitoring and care for asthmatic patients management from Lahore city of Pakistan. National Journal of Medical and Health Sciences. 2021; 3(2): 49-58.

[6] Ghani MU, Sabar MF, Shahid M, Awan FI, Akram M. A report on asthma genetics studies in Pakistani population. Advancements in Life Sciences. 2017 Feb; 4(2): 33-8.

[7] Kuruvilla ME, Lee FE, Lee GB. Understanding asthma phenotypes, endotypes, and mechanisms of disease. Clinical Reviews in Allergy & Immunology. 2019 Apr; 56: 219-33. doi: 10.1007/s12016-018-8712-1.

[8] Khan MA. Monthly and seasonal prevalence of asthma and chronic obstructive pulmonary disease in the District Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan. The Egyptian Journal of Bronchology. 2022 Dec; 16(1): 63. doi: 10.1186/s43168-022-00166-2.

[9] Jackson DJ, Sykes A, Mallia P, Johnston SL. Asthma exacerbations: origin, effect, and prevention. Journal of Allergy and Clinical Immunology. 2011 Dec; 128(6): 1165-74. doi: 10.1016/j.jaci.2011.10.024.

[10] Holgate ST. Pathogenesis of asthma. Clinical & Experimental Allergy. 2008 Jun; 38(6): 872-97. doi: 10.1111/j.1365-2222.2008.02971.x.

[11] Khazdair MR, Ghorani V, Alavinezhad A, Boskabady MH. Pharmacological effects of Zataria multiflora Boiss L. and its constituents focus on their anti-inflammatory, antioxidant, and immunomodulatory effects. Fundamental & Clinical Pharmacology. 2018 Feb; 32(1): 26-50. doi: 10.1111/fcp.12331.

[12] Khazdair MR, Ghorani V, Alavinezhad A, Boskabady MH. Effect of *Zataria multiflora* on serum cytokine levels and pulmonary function tests in sulfur mustard-induced lung disorders: A randomized double-blind clinical trial. Journal of Ethnopharmacology. 2020 Feb; 248: 112325. doi: 10.1016/j.jep.2019.112325.

[13] Sajed H, Sahebkar A, Iranshahi M. *Zataria multiflora* Boiss.(Shirazi thyme)—an ancient condiment with modern pharmaceutical uses. Journal of Ethnopharmacology. 2013 Feb; 145(3): 686-98. doi: 10.1016/j.jep.2012.12.018.

[14] Hosseinzadeh H, Ramezani M, Salmani GA. Antinociceptive, anti-inflammatory and acute toxicity effects of *Zataria multiflora* Boiss extracts in mice and rats. Journal of Ethnopharmacology. 2000 Dec; 73(3): 379-85. doi: 10.1016/S0378-8741(00)00238-5.

[15] Karimi A, Krähmer A, Schulz H, Meiners T. Variation of secondary metabolite profile of *Zataria multiflora* Boiss. populations linked to geographic, climatic, and edaphic factors. Frontiers in Plant Science. 2020 Jul; 11: 508548. doi: 10.3389/fpls.2020.00969.

[16] Zamani N, Shams M, Nimrouzi M, Zarshenas MM, Foroughi AA, Abarghooei EF *et al.* The effects of *Zataria multiflora* Boiss.(Shirazi thyme) on nonalcoholic fatty liver disease and insulin resistance: A randomized double-blind placebo-controlled clinical trial. Complementary Therapies in Medicine. 2018 Dec; 41: 118-23. doi: 10.1016/j.ctim.2018.09.010.

[17] Mahmoodi M, Koohpeyma F, Saki F, Maleksabet A. The protective effect of *Zataria multiflora* Boiss. hydroalcoholic extract on TNF-α production, oxidative stress, and insulin level in streptozotocin-induced diabetic rats. Avicenna Journal of Phytomedicine. 2019 Jan; 9(1): 72.

[18] Mahboubi M. Management of acute cough by *Zataria multiflora* Boiss as an alternative treatment. Journal of Integrative Medicine. 2018 Jan; 16(1): 20-5. doi: 10.1016/j.joim.2017.12.006.

[19] Mortazavi Moghaddam SG, Kianmehr M, Khazdair MR. The possible therapeutic effects of some medicinal plants for chronic cough in children. Evidence-Based Complementary and Alternative Medicine. 2020 Oct; 2020. doi: 10.1155/2020/2149328.

[20] Alavinezhad A, Hedayati M, Boskabady MH. The effect of *Zataria multiflora* and carvacrol on wheezing, FEV1 and plasma levels of nitrite in asthmatic patients. Avicenna Journal of Phytomedicine. 2017 Nov; 7(6): 531.