

Comparison of Berberine and Dexamethasone on Blood and Bronchial Inflammatory Cells of Ovalbumin Sensitized Guinea Pigs

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Abstract

Objective: To compare the anti-inflammatory effects of berberine and dexamethasone in ovalbumin sensitized guinea pigs.

Methods: This experimental controlled study was conducted in April, 2016 at Postgraduate Medical Institute, Lahore. Twenty-four healthy guinea pigs were selected for study. Six of these were assigned randomly in each group; normal control, ovalbumin (OVA) sensitized, berberine treated and dexamethasone treated groups. Airway inflammation was induced on day 0 and 14 by OVA injections via peritoneal route and by inhalation on 25th, 26th and 27th day in each group excluding the normal control. Berberine (1.8 mg/kg) and dexamethasone (20 mg/kg) were introduced via peritoneal route 30 minutes earlier to each trial in berberine treated and dexamethasone treated groups respectively.

Results: Total leukocyte count (TLC) in blood sample of berberine treated group ($9990 \pm 1346 \text{ mm}^3$) and dexamethasone treated group ($9054 \pm 1432 \text{ mm}^3$) was significantly low than OVA sensitized group ($14261 \pm 3151 \text{ mm}^3$). TLC of broncho-alveolar lavage (BAL) fluid in berberine treated group ($384 \pm 26 \text{ mm}^3$) and dexamethasone treated group ($306 \pm 86 \text{ mm}^3$) was significantly low than OVA sensitized group ($598 \pm 110 \text{ mm}^3$). Eosinophil percentage in blood of berberine treated (21.50 ± 3.08) and dexamethasone treated (13.33 ± 5.65) groups were significantly low than OVA sensitized group (30.33 ± 6.74), while eosinophil percentage in BAL fluid was 26.00 ± 6.69 and 21.00 ± 7.46 in berberine treated and dexamethasone treated groups respectively with significant difference from OVA sensitized group value of 40.00 ± 7.79 .

Conclusion: Berberine and dexamethasone both had reduced TLC and eosinophil percentage in both blood and BAL fluid as compared to OVA sensitized group but berberine is less effective than dexamethasone.

Keywords: berberine, dexamethasone, asthma, airway inflammation

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Introduction

Asthma is a common reversible chronic inflammatory condition of the lungs that leads to narrowing of the airways. Common symptoms

include breathlessness, wheezing, chest tightness and coughing. In 2016, it was estimated that 339.4 million people worldwide were affected by asthma.¹ In Pakistan, asthma is also prevailing very rapidly. Almost two million people are experiencing asthma per year and still 5% increase in cases is noted every year.²

The known risk factors for developing asthma include a combination of genetic predisposition and environmental exposure to various substances such as tobacco smoke and chemical irritants that may provoke allergic reactions or irritate the airways. Airways inflammation causes changes in the geometry and biomechanical properties with excessive production of mucus and consequent clogging and decreased lumen, resulting

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in development and persistence of airflow obstruction, predominantly in the early morning or evening periods.³ Multiple mechanisms are suggested for the production of this disease but the inflammation of smaller airways is the main feature. There is hyper-responsive of immune system to the innocuous stimuli. Asthma is exploited by multiple cellular elements and immune cells which include T lymphocytes, mast cells, macrophages, neutrophils, epithelial cells and eosinophils. Eosinophils play an important role in asthma pathogenesis, and eosinophilia is a typical hallmark of asthma in humans.⁴

Corticosteroids are considered to be the most potent and effective anti-inflammatory medications currently available for the symptomatic control and maintenance of atopic or non-atopic asthma, as recommended by GINA (global initiative for asthma) guidelines.⁵ Use of steroids is associated with a number of serious adverse effects. The loss of corticosteroid efficacy is an important issue in severe asthma management and may lead to poor asthma control and deterioration of airflow.⁶

Many people prefer to use different kinds of herbs for their treatment due to their folk knowledge and dislike conventional medicine because of their higher cost, adverse effects and reduced effectiveness.⁷ Impressively, recent studies have shown that berberine exerts anti-inflammatory and anticancer effects.⁸ In one study, berberine dose-dependently reversed the alterations induced by ovalbumin (OVA) in the asthmatic rats.⁹ There is scarce scientific research to evaluate the possible role of berberine in limiting the allergy induced inflammation in diseases like asthma. This study was planned with the objective to find out the possible anti-inflammatory role of berberine in asthma and its comparison with dexamethasone in OVA sensitized guinea pigs.

Methods

This is an experimental study which was conducted in April, 2016 at Pharmacology department of Post Graduate Medical Institute (PGMI), Lahore. Permission was obtained from research ethical committee for basic science of institute. Total twenty-four healthy guinea pigs of either gender weighing 340-500 gm were purchased from Lahore Zoo. (For one week, these animals were looked after at 22-24°C temperature for adaptation to the environment in PGMI. They were provided with plenty of water and food. The animals were checked for any sign of disease for exclusion from study.

After initial selection, they were assigned randomly to four groups by using lottery method. Airway inflammation was induced in animals of 3 groups excluding the normal control group as shown in table-1. The chemical used during study were ovalbumin (Sigma Aldrich, Poole U.K), alum (Biosector, Denmark), phosphate buffer saline (PBS) (Sigma Aldrich, Germany), berberine chloride (Sigma, USA), dexamethasone (OBS Pharma Pakistan) and chloroform (Scharlab S.L. European Union).

Blood sample was taken on 28th day of study by cardiac puncture while the animal was given light chloroform anesthesia.¹³ After blood sampling, guinea pigs were sacrificed with cervical dislocation. Bronchoalveolar lavage (BAL) fluid was collected by initially infusing and then withdrawing of 5 ml ice cold PBS through a cannula in the trachea and lungs.¹⁴

Total leukocyte count (TLC) was computed manually on Neubauer chamber both for blood and BAL fluid samples. Assessment of differential leucocyte count (DLC) was done with the help of Giemsa stained blood film under the oil immersion lens both for blood and BAL fluid samples.

Table 1: Allergic Airway Inflammation Induction and Drug Treatment

Groups	Sensitization on day 0 and 14	Intranasal challenge on day 25,26,27	Treatment on day 25,26,27
Normal control	1.5ml PBS by intraperitoneal route	PBS	Distilled water (DW) (5ml/kg) by intraperitoneal route half an hour before challenge
Ovalbumin sensitized	0.5ml OVA (100µg) + 1 ml alum (200mg) in PBS by intraperitoneal route ¹⁰	1% OVA in PBS	DW (5ml/kg) by intraperitoneal route half an hour before challenge
Berberine treated	0.5ml OVA (100µg) + 1 ml alum (200mg) in PBS by intraperitoneal route	1% OVA in PBS	Berberine (1.8mg/kg) ¹¹ by intraperitoneal route half an hour before challenge
Dexamethasone treated	0.5ml ovalbumin (100µg) + 1 ml alum(200mg) in PBS by intraperitoneal route	1% OVA in PBS	Dexamethasone (20 mg/kg) ¹² by intraperitoneal route half an hour before challenge

Data was entered and analyzed by using SPSS 20 software. After checking normal distribution, TLC and DLC were described as mean±standard deviation.

Table 2: Comparison of Blood and BAL Sample TLC (Mean±SD) in Study Groups (n=6)

Group=n	Blood	BAL
	Mean±SD (mm ³)	Mean ±SD (mm ³)
Normal control	8578.83±2065.65**	190.00±21.35**
Ovalbumin sensitized	14261.67±3151.36	598.66±110.94
Berberine treated	9990.33±1346.53*	#
Dexamethasone treated	9054.83±1432.29**	384.00±26.83**
ANOVA	<0.001	<0.001

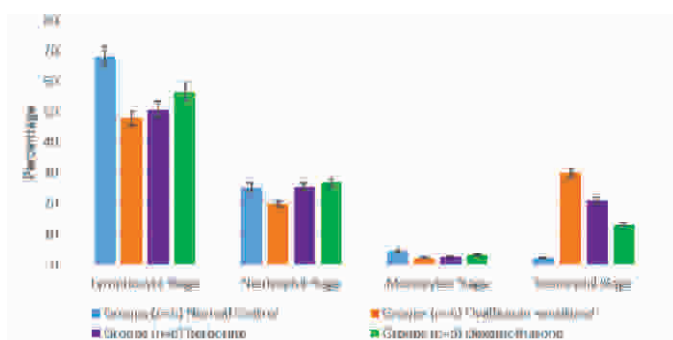
*p value ≤ 0.05, **p value ≤ 0.001 vs ovalbumin sensitized, #p value ≤ 0.001 vs normal

ANOVA was applied to compare TLC and eosinophil % of blood and BAL fluid. The group mean difference was detected by applying the post hoc Tukey's test. Statistically significant p-value was regarded as ≤ 0.05.

Results

The TLC in blood and BAL fluid samples of OVA sensitized group was markedly higher as compared to normal control. Berberine and dexamethasone treated animals had significant lower blood and BAL fluid TLC as compared to OVA sensitized group and insignificant higher than normal control (table-2)

Figure-1: Cluster Bar Chart Showing DLC in Blood



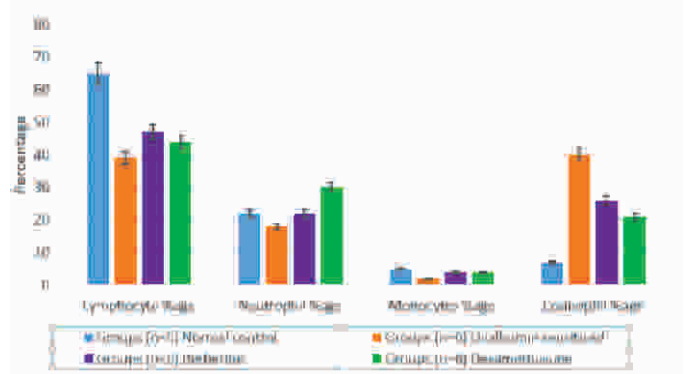
Sample of Study Groups

Figure-1 and figure-2 show DLC of blood and BAL fluid

Eosinophilic percentage in blood as well as BAL fluid was significantly higher in OVA sensitized group as compared to normal control. Berberine and dexamethasone treated animal had significantly lower eosinophilic percentage as compared to OVA sensitized control but higher as compared to normal control, with significantly less percentage in blood but not BAL fluid of dexamethasone group as

compared to berberine group (table-3).

Figure -2: Cluster Bar Chart Showing BAL Fluid



DLC in Study Groups

Discussion

Herbal plants have gained fame due to their cultural acceptance, lack of expense, minimum adverse effect and drug resistance.¹⁵ The berberine was selected in this study to find out its competency as an anti-inflammatory agent in the disease process of asthma in the guinea pigs which were sensitized with OVA. Then these results were compared with that of dexamethasone. The reason for selection of guinea pig as

Table 3: Comparison of Blood and BAL Sample Eosinophilic Percentage in Study Group

Group n=6	Blood Eosinophil %	BAL Eosinophil %
	Mean±SD (mm ³)	Mean ±SD (mm ³)
Normal Control	2.16±2.14**	7.5±4.9**
Ovalbumin Sensitized	30.33±6.74	40.00±7.79
Berberine	#	#
Dexamethasone	21.50±3.08*	26.00±6.69**
ANOVA	<0.001	<0.001

*p value ≤ 0.05, **p value ≤ 0.001 vs ovalbumin sensitized, #p value ≤ 0.001 vs normal, ° p value ≤ 0.05 vs dexamethasone

experimental animal was due to their similarities in the airways structure and disease process of asthma in human beings.

Our study demonstrates higher blood TLC in OVA sensitized group when compared with normal control group and difference was statistically significant with p-value 0.001. The results are complemented by the observations made by Arora et al (2016) in their study, of increased blood TLC levels in disease group as compared to the normal group.¹⁶

The decreased blood TLC levels were observed in berberine treated group as compared to OVA sensi-

zed group but these values were higher than normal control group. The similar effects were produced by berberine in a study conducted by Mahajan and Mehta, 2011.¹⁷ In our study, highly significant (p-value 0.001) lower levels of blood TLC were exhibited in dexamethasone treated group in comparison to OVA sensitized group. Arora et al (2016) and Murad and Hassnain (2014) have also revealed similar results after dexamethasone administration in murine model of asthma and OVA sensitized guinea pigs with p-values of 0.01 and 0.05 respectively when matched with sensitized groups.^{16,18} There is no study to compare the effect of berberine on blood TLC.

According to the results of current study, high count of white blood cell was noted in BAL after sensitization with OVA. The number of white blood cells was decreased more in both the treatment groups as compared to the group sensitized with OVA having a p-value of 0.001. In a rat model of asthma, orally administered berberine lowered BAL fluid TLC with p-value < 0.05.⁹ Difference in significance of results may be due to difference in route of administration and animal species.

Eosinophils play important role in asthma pathogenesis and this study demonstrates higher level of eosinophil percentage in blood and BAL samples of OVA sensitized group as compared to berberine treated group with a significant p-value of 0.05. Similar results were expressed in a study conducted by Mahajan and Mehta (2010) after treatment with β sitosterol compound from herb *Moringa oleifera*.¹⁷ The ratio of eosinophil in blood was significantly lesser (with p-value 0.001) in dexamethasone treated group when compared with ova sensitized group. These results are in accordance with the findings (with p-value 0.001) of Naik et al. (2013) study.¹⁹ The less significant levels (p-value 0.05) of TLC and eosinophil in BAL fluid were observed in similar asthma model with smaller doses of dexamethasone by Murad and Hassnain (2014).¹⁸

Overall impression of this study is that berberine and dexamethasone treatments have decreased the count of total leukocytes and eosinophils in blood as well as BAL fluid samples but berberine is less effective than dexamethasone.

This study is limited to evaluation of basic markers of allergic inflammation. Literature is available for possible role of berberine as suppressant of mast cell

degranulation²⁰ and inhibitor of cytokine production²¹ including interleukins²² which are involved in pathophysiology of asthma. Further studies on effect of berberine on airway hyperresponsiveness will confirm its role in prevention or treatment of allergic airways diseases.

Conclusion

Berberine is an effective anti-inflammatory agent in allergic asthma. These findings reveal the fruitful use of berberine to mitigate inflammatory process causing worsening of asthma.

Conflict of interest

None

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Authors Contribution

- CS:** Conceptionlization of Project
ZTS: Data Collection
SA: Literature Search
MM: Statistical Analysis
SJ: Drafting, Revision
KR: Writing of Manuscript