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Effect of Deer Velvet Antler on Airway Inflammation in Ovalbumin Sensitized Guinea Pigs

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ABSTRACT

Background: Despite better understanding of asthma pathology and adherence to recommended treatments still many patients are experiencing uncontrolled asthma. Deer velvet antler is a traditional animal-based medicine which has shown many pharmacological effects in various diseases including asthma. **Objective:** To evaluate the effect of deer velvet antler on airway inflammation in ovalbumin sensitized guinea pigs and to compare it with that of dexamethasone. **Study Design:** Comparative study. **Duration:** 12 weeks. **Methodology:** Animals were placed in four groups with six in each. Animals in group I and II were taken as negative and positive control and animals in groups III and IV were treated with deer velvet antler powder and dexamethasone respectively along with induction of allergic airway inflammation by ovalbumin sensitization on day 0 and 14 and challenge on day 25, 26 and 27. On day 28, blood and bronchoalveolar lavage (BAL) fluid samples were taken for total leukocyte count and eosinophil percentage. **Results:** The study reported that total leukocyte count and eosinophil percentage in blood and bronchoalveolar lavage fluid of deer velvet antler treated guinea pigs was significantly reduced as compared to asthma control, but in comparison with dexamethasone difference was not significant. **Conclusion:** Deer velvet antler reduces allergic airway inflammation supporting the hypothesis of study.

Keywords: Deer velvet antler, Allergic airway inflammation, Guinea pigs, Dexamethasone.

INTRODUCTION

Bronchial asthma is a chronic airway disorder in which airways are inflamed and there is severe eosinophilic and lymphocytic infiltration, overproduction of mucus, hyper- responsiveness of airways, and reversible airway obstruction.[1] According to WHO, an estimated 300 million people of all ages are suffering from asthma, and approximately 250,000 people die from asthma every year.[2] Asthma has been divided into two categories: Atopic asthma initiated by inhalation of an allergen, which leads to production of Ig-E antibodies and Non-atopic asthma occurs in individuals who are non-allergic and it is non Ig-E mediated.[3]

Despite better understanding of asthma pathology and adherence to recommended treatments still many patients are experiencing uncontrolled asthma, these medicines especially steroids on chronic use develop serious side effects. These factors are compelling for new researches to be done and find alternative treatments with good efficacies Deer velvet antler (DVA) has had great medicinal value in China for over 2000 years. In the Eastern population, it is mainly used to strengthen the body and as healing and preventive therapy. Deer velvet antlers are formed yearly on protuberances known as pedicles, present primarily on the frontal bone of the skulls of male deer.

Many inorganic and organic substances are present in DVA including calcium, amino acids, proteins, carbohydrates, polysaccharides, epidermal growth factor, insulin like growth factor, hormones etc.[4]

Regarding role of deer velvet antler in asthma, immunomodulatory effects of DVA have been reported in other studies. Aqueous extract of DVA has played a role in inhibiting T-cell proliferation, differentiation, and cytokine secretion after antigenic stimulation in vitro. It has also inhibited the release of IL-β1 and TNF-α from macrophages in mice.[5] Kim *et al*, (2004) reported the suppression of pro-inflammatory cytokines.

It has been demonstrated that even oral administration of aqueous extract of DVA has immunosuppressive activity. Moreover, the digestive process did not affect the immune-regulating peptides.[7] It has inhibitory effects on the expression of many cytokines (IL-2, TNF-α, IL-12 and IFN-γ) which are involved in pathogenesis of different TH2 mediated disease including asthma.[8] Kuo *et al*, (2012) demonstrated that prevention and alleviation of asthmatic symptoms after administration of DVA depend on elevation of Th1 and Treg cytokines and inhibition of Th2 and Th17 cytokines and Ig-E production and is able to regulate Th1/Th2 balance. DVA demonstrated complex effects on the body's immune system as they inhibits B cell synthesis and stimulates the generation of T and NK cells and will play disparate roles in the defense against different diseases.[10]

The role of DVA to protect and control allergic diseases like asthma has not been extensively studied. So, this study was designed to provide scientific basis for traditional use of DVA and to compare its anti-inflammatory effects with dexamethasone.

METHODOLOGY

In this study, guinea pigs were preferred to create a model of allergic bronchial asthma because, anatomy of airways and response to inflammatory mediators resemble humans. Also, guinea pigs have strong ability to develop direct anaphylactic bronchoconstriction after antigen challenge.[11] Healthy male guinea pigs weighing 350 to 490 g were included. Animals were kept in the animal house of Post Graduate Medical Institute for one week for acclimatization at temperature of 22-24℃, kept under natural light and dark cycle and were fed with food (vegetables, fruits and chick pea) and water. Animals were numbered from 1 to 24 and assigned randomly to groups I, II, III and IV, 6 in each group, by simple lottery method.

Treatment Protocol: Animals were divided into four groups as **Group I:** NC (Negative Control), **Group II:** PC (Positive Control), **Group III:** DVA, **Group IV:** DEXA.

Groups	Intraperitoneal Sensitization days: 0 and 14	Airway Challenge Days: 25, 26, 27	
		1hr before each airway challenge (orally)	Airway Challenge
Group I (Negative Control)	Phosphate buffer saline (0.5ml)	Gum tragacanth dissolved in distilled water (4.2ml)	Phosphate buffer saline
Group II (Positive Control)	Ovalbumin (0.5ml)	Gum tragacanth dissolved in distilled water (4.2ml)	1% ovalbumin
Group III (DVA)	Ovalbumin (0.5ml)	DVA (4.2 ml)	1% ovalbumin
Group IV (DEXA)	Ovalbumin (0.5ml)	DEXA (4.2ml)	1% ovalbumin

Deer velvet antler (Herb show Bio-technology co., Ltd Shangai, China) was in the form of thin slices which were ground into fine powder by means of pulverizing machine and stored at 4℃.

Induction of allergic airway inflammation was performed by two intraperitoneal injections of ovalbumin along with aluminium hydroxide followed by airway challenge with ovalbumin.[12]

Ovalbumin is derived from chicken egg and is a frequently used allergen that induces a robust, allergic pulmonary inflammation in laboratory rodents. The ova induced asthmatic model is a widely used model that results in characteristics features of asthma allowing the study and assessment of novel treatments.[13] Aluminium hydroxide has been used along with ovalbumin as an adjunct to elicit Ig-E antibodies production.[14] The intraperitoneal route of administration is preferred for sensitization, because Ig-E production is more when given by intra-peritoneal route as compared with other routes.[15]

Animals in group 1 were sham-sensitized and challenged with PBS only. Animals in group II, III and IV were sensitized on day 0 and 14 by intraperitoneal injection of 100µg of ovalbumin with 20 mg of aluminum hydroxide (Biosector Denmark) in phosphate buffer saline (PBS).[12] Airway challenge was achieved with 1% ovalbumin in PBS solution through nasal inhalation. Each animal was nebulized for 20 seconds on days 25, 26 and 27.[15] Deer velvet antler (DVA) powder and dexamethasone powder suspensions were prepared by using gum tragacanth as suspending agent. Animals in group III and IV were administered deer velvet antler (DVA) in dose of 190 mg/kg and dexamethasone 20mg/kg of body weight respectively while animals in group 1 and 2 were given vehicle only.[16] Each compound was administered by oral route as a single morning dose one hour before ovalbumin challenge.

At day 28 (24 hour after last challenge), 3ml blood was withdrawn via cardiac puncture under light anesthesia and placed in ethylenediamine tetra acetic acid (EDTA) vacutainers for estimation of total and differential cell count (TLC, DLC).[17]

After taking blood samples, animals were sacrificed under anesthesia. Lungs were lavaged three times by instillation and withdrawal of 5ml ice cold PBS through trachea and BAL fluid was collected.[18]

Variables used for the assessment of allergic airway inflammation are total leukocyte count of blood, eosinophil % of blood, total leukocyte count of bronchoalveolar lavage (BAL) fluid and eosinophil % of BAL fluid

Calculation of TLC: Total leukocyte count was calculated manually

Calculation of Eosinophil percentage: Thin blood film was stained with Giemsa dye and under oil immersion

lens of microscope, 200 cells were counted and % of eosinophil cell type was calculated.

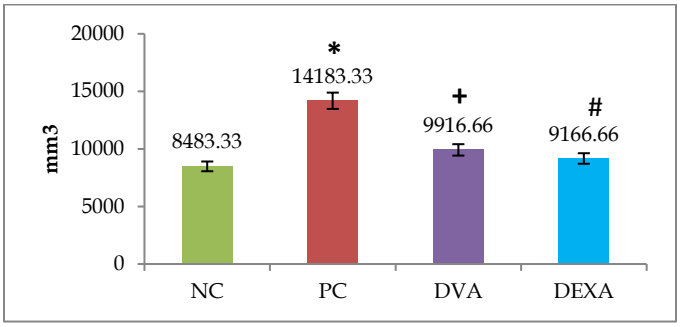
Data Analysis: Data was entered and analyzed by using SPSS 15 software. As the data was normally distributed, it was presented Mean and compared among groups by using One Way ANOVA and post hoc Tukey s test. *p* value ≤ 0.05 was considered statistically significant.

RESULTS

Total Leukocytes Count of Blood

The total leukocyte count of blood (Mean ± SD) was 8483.33±2056.61, 14183.33±3284.76, 9916.66±1393.43 and 9166.66±1406.65 /mm^3 in negative control, positive control, DVA treated group and DEXA treated group respectively. When comparison was made by ANOVA the difference was significant with *p* value 0.01 (Fig.1).

Fig 1: Effect of deer velvet antler and dexamethasone on total leukocyte count (Mean ± SE) of blood of ovalbumin sensitized guinea pigs (n=6)

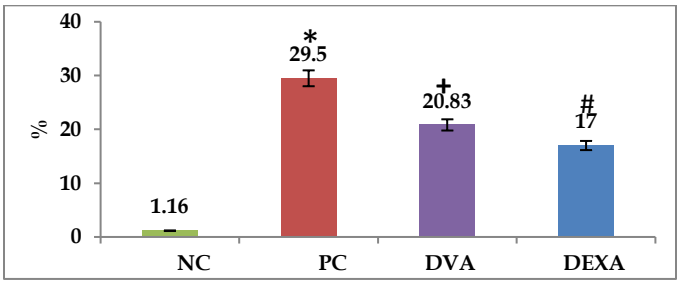


** p value ≤ 0.001 vs NC, + p value ≤ 0.05 vs PC and # p value ≤ 0.01 vs PC NC= Negative control, PC=Positive control, DVA=Deer Velvet Antler treated group, DEXA= Dexamethasone treated group*

Eosinophil Percentage of Blood:

The eosinophil percentage (Mean ± SD) of blood was 1.16 ± 1.17, 29.5 ± 6.25.20, 83 ± 3.31 and 17.00 ± 5.65 % in negative control, positive control, DVA group and DEXA group respectively. When comparison was made by ANOVA the difference was significant with *p* value < 0.001 (Fig.2).

Figure 2: Bar chart showing effect of deer velvet antler and dexamethasone on eosinophil percentage (Mean ± SE) of blood of ovalbumin sensitized guinea pigs(n=6)

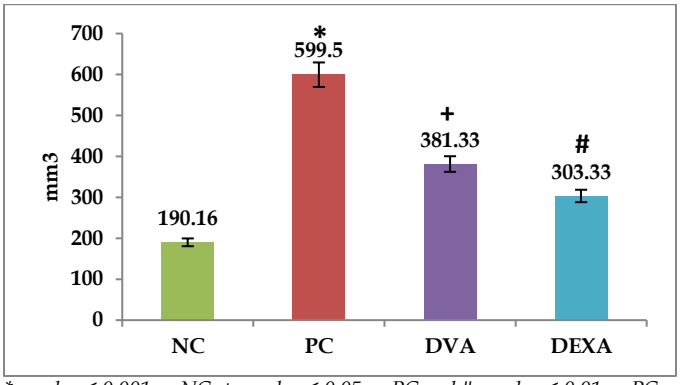


** p value ≤ 0.001 vs NC, + p value ≤ 0.05 vs PC and # p value ≤ 0.01 vs PC NC= Negative control, PC=Positive control, DVA=Deer Velvet Antler treated group, DEXA= Dexamethasone treated group*

Total Leukocyte Count of BAL Fluid:

The total leukocyte count (Mean ± SD) of BAL fluid was 190.16 ± 24.44, 599.50 ± 109.66, 381.33 ± 30.14 and 303.33 ± 86.17/mm^3 in negative control, positive control, DVA group and DEXA group respectively. When comparison was made by ANOVA the difference was significant with *p* value < 0.001 (Fig. 3).

Figure 3: Bar chart showing effect of deer velvet antler and dexamethasone on total leukocyte count (Mean±SE) of BAL fluid of ovalbumin sensitized guinea pigs (n=6)

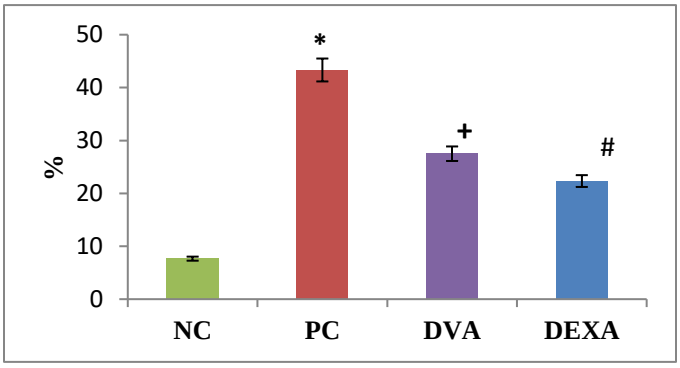


** p value ≤ 0.001 vs NC, + p value ≤ 0.05 vs PC and # p value ≤ 0.01 vs PC NC= Negative control, PC=Positive control, DVA=Deer Velvet Antler treated group, DEXA= Dexamethasone treated group*

Eosinophil Percentage of BAL fluid

The eosinophil percentage (Mean±SD) of BAL fluid was 7.66 ± 5.27, 43.33 ± 8.18, 27.50 ± 6.37 and 22.33 ± 7.65 in negative control, positive control, DVA group and DEXA group respectively. When comparisons were made by ANOVA the difference was significant with *p* value < 0.001 (Fig.4)

Figure 4: Effect of deer velvet antler and dexamethasone on eosinophil percentage (Mean ± SE) of BAL fluid of ovalbumin sensitized guinea pigs (n=6)



** p value ≤ 0.001 vs NC, + p value ≤ 0.05 vs PC and # p value ≤ 0.01 vs PC NC= Negative control, PC=Positive control, DVA=Deer Velvet Antler treated group, DEXA= Dexamethasone treated group*

DISCUSSION

All the available treatments for asthma have limitations due to hazardous adverse effects and failure of response

hence this study was designed to evaluate the potential of DVA to prevent and control asthma

In this study, total leukocyte count and eosinophil percentage in blood and BAL fluid were used as allergy indicators. Eosinophils play an important role in the pathology of late asthmatic reactions. They secrete dangerous granular proteins, cytokines, and chemokines which lead to airway inflammation, hypersecretion of mucus, smooth muscle contraction and remodeling of airways.[19] Total leukocyte level is increased in asthma due to increase inflammatory reaction. Neutrophils have a role in establishing severe chronic asthma, in the onset of a fatal acute episode of asthma[20] and it is believed that increased expression of Th2 cells plays a significant role in the pathogenesis of atopic asthma.[21]

In this study after DVA treatment, results show significant reduction in total leukocyte count and eosinophil % in blood and BAL fluid. Comparison between DVA and dexamethasone treated group was not statistically significant. Hence both treatment groups have comparable effects which is the most significant result of this study.

Kuo et al., studied effects of DVA extracts on asthmatic mouse model by daily oral administration of DVA (10mg/day for 30 days) and observed that eosinophil count of BAL fluid was significantly decreased. In DVA treated group as compared to sensitized group with p value 0.001which is significantly higher than the result of present study with p value 0.01. This difference is may be due to differences in the duration of treatment as DVA powder (190mg/kg) was administered only on three days in present study.

As limited research work has been conducted on use of DVA for asthmatic prevention and control hence results of this study are compared with other natural products to show its worth.

Findings of present study are comparable to results of other traditional herbs investigated for use in asthma treatment. Aqueous extract of *Urtica dioica* was investigated in the asthmatic rat model. It significantly ($p < 0.01$) decreased eosinophilic infiltration in BAL fluid (−60%), the levels of leucocytes (−32.75%) and lymphocytes (−29.22%) in serum, and so effectively decreased recruitment of inflammatory cells.[22]

In another study, it was found that Kangzhi syrup effectively inhibited the infiltration of total inflammatory cells ($p < 0.0001$) and reduced the percentage of eosinophil in BAL Fluid ($p < 0.0001$) in ovalbumin induced cough variant asthma (CVA) in guinea pigs.[23]

CONCLUSION

This research demonstrates that DVA is able to control allergic airway inflammation after oral administration and results are comparable to those of dexamethasone.

LIMITATIONS

Limited no. of parameters were calculated and possible mechanism of action of DVA could not be determined.

CONFLICT OF INTEREST / DISCLOSURE

None.

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