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Cassia Angustifolia; A Potential Anti-Inflammatory Agent in Allergic Asthma in Rats

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Author`s Contribution

[1,2]Substantial contributions to the conception or design of the work or the acquisition, methodology, analysis, [5]Final approval of the version to be published. [2,3,4]Active Participation in active, Drafting the work or revising it critically for important intellectual content

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ABSTRACT

Objective: To identify anti-inflammatory effects of Cassia angustifolia in allergic airway disease in comparison to emodin and dexamethasone.

Methodology: This experimental study was conducted at animal house of Postgraduate Medical Institute, Lahore, from February 2022 to March 2022 in which 42 male Sprague Dawley rats were divided into 7 groups having 6 rats in each group. Group I (Normal control group), Group II (Diseased control group), Group III (Dexamethasone Control group), Group IV (Emodin Control group), Group V, VI, and VII were low, medium and high dose of Cassia angustifolia leaf ethanolic extract treated groups. Asthma was induced in all rats except the rats of normal control group at the start of the study by sensitizing rats with intraperitoneal injection of Ovalbumin on day 0 and 14. They were challenged with nebulization of 1% w/v ovalbumin solution on days 22, 23 and 24. Respective drugs were given from day 15 to 24 as twice oral daily doses. Blood samples were taken at day 25 via cardiac puncture. TLC, eosinophil count, IgE antibodies and IL-13 were measured using commercially available kits.

Results: High-dose Cassia angustifolia demonstrated significant anti-inflammatory effects comparable to emodin and dexamethasone, effectively reducing TLC, eosinophil count, IgE levels, and IL-13 levels. Low and medium doses of Cassia angustifolia showed weaker anti-inflammatory effects, not significantly reducing TLC or eosinophil counts compared to the disease control group. All treatment groups exhibited significantly lower IgE and IL-13 levels than the disease control, indicating the anti-allergic and anti-inflammatory potential of Cassia angustifolia.

Conclusion: Cassia angustifolia, particularly at high doses, exhibits anti-inflammatory effects similar to standard treatments like emodin and dexamethasone, suggesting its potential as an alternative therapy for allergic asthma. However, it does not surpass the efficacy of dexamethasone in controlling specific inflammatory pathways. Further studies are warranted to explore its therapeutic application in asthma management.

Keywords: Dexamethasone, Cassia angustifolia, emodin, allergic asthma

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Introduction

Asthma is a major public health problem with huge socioeconomic burden. Deaths from asthma approach 0.40 million (0.36 million to 0.44 million) in 2015.[1] It is a chronic disease that affects the airways of lungs. Pathophysiology of asthma involves mucosal edema , swelling, mucus secretion in the airways, and contraction of smooth muscles of airways (bronchospasm). Symptoms of asthma include dry cough, wheezing, dyspnea and chest

tightness. Shortness of breath which may occur with activity or even at rest. Allergies, respiratory infections, stress, exercise, and medications are some causative factors of bronchial asthma.[2]

Allergic asthma also called asthma mediated type 2 inflammation is associated with high level of IgE and high eosinophil count and affects almost 50-80 % of asthmatic patients. There is increase of T-helper 2 (Th2) cytokines, mainly of IL-13 along with IL-4 IL-5 and innate lymphoid cells type 2 (ILC2) immune cells resulting in eiosinophilic airway infiltration.[3]

Standard treatment of asthma includes $beta_2$ agonists, inhaled plus oral corticosteroid and leukotriene receptor antagonists.[4] The adverse effects of these drugs limit their usefulness so there is always a room for alternative medicines in the treatment of asthma.

Cassia angustifolia is a traditional ayurvedic edible shrub containing anthraquinones (AQs) as the principle active constituents. There are antibacterial, hepato-protective, hypo-chlosterolaemic, anti-diabetic, antioxidant, and anti-inflammatory properties associated with the *Cassia angustifolia* plant.[5] Ripe Cassia pods and leaves include aglycone and glycoside anthraquinones like rhein, aloe-emodin, chrysophanic acid, or sennosides.[6] Emodin is also present in *Cassia angustifolia*. It has immunosuppressive, anti-atherosclerotic, anti-microbial and anti-inflammatory properties.[7] So, it can be used for the treatment of chronic inflammatory conditions like allergic airways.

The aim of study was to identify anti-inflammatory effects of *Cassia angustifolia* in allergic airway disease in comparison to emodin and dexamethasone by determining serum levels TLC, eosinophils, IgE and IL-13 so that natural substances could be added as adjuvant therapy for asthma.

Methodology

This experimental study of 25 days duration was conducted at animal house of Postgraduate Medical Institute, Lahore from February to March 2022 after approval by Ethical Committee of Postgraduate Medical Institute, Lahore.

For this project, Forty two adult healthy male Sprague Dawley rats weighing (200-250 grams) were purchased from local market of Lahore. Rats showing signs of any illness were excluded from the study. The rats undergone one week acclimatization prior to any experimentation. They were placed in respective cages at standard room temperature (22-24ºC) with proper ventilation. All the animals were given access of rat chow and water. After one week, all rats were divided into seven groups by simple random method by ballotting having 6 rats in each group. Group I served as normal control group, group II as diseased control group, group III was dexamethasone control group, group IV as emodin control group and groups V, VI, and VII were treated with low, medium and high dose of *Cassia anugustifolia* leaf ethanolic extract respectively.

Asthma was induced in all the rats except normal control by ovalbumin (Sigma). Ovalbumin solution was prepared by mixing ovalbumin 1mg with 50 mg aluminum hydroxide mixed in 1 ml of phosphate buffer saline (PBS) solution.[9] Sensitization was done on day 0 and 14 of the experiment by giving intraperitoneal injections of 1 ml this ovalbumin solution. Afterwards they were challenged on days 22, 23 and 24 by inhalational method through nebulization for half hour using 1% w/v ovalbumin.[10]

Dexamethasone (sigma) and emodin (AmBeed) were given to group III and IV in doses of 2.5 mg/kg [8] and 10 mg/kg[10] as twice daily doses through oral gavage.

Cassia angustifolia leaves were purchased from local herb market. They were washed and air dried in shadow. They were coarsely ground and soaked into 80% ethanol for 3 days. Ethanol was then filtered with Whatman filter paper no 1 and the filtrate was dried in rotary evaporator to a thick creamy paste. This extract was administered in group V, VI and VII in a dose of 200 mg/kg, 400 mg/kg and 800 mg/kg respectively from day 15 to 24 of study twice daily through oral gavage.[8]

TLC and eosinophil count were measured in blood drawn through cardiac puncture and saved in EDTA containing vials on day 25 of experiment. Neu bar chamber was used to find these values.

Blood was drawn through cardiac puncture and saved in gel vacutainers. Serum was separated with help of centrifuge. IgE levels were measured through ELISA technique using kit made by Glory Science company, USA.

IL-13 levels were measured in separated serum through ELISA technique. Kits made by Glory Science Company; USA were used for this purpose.

Data were entered and analyzed in SPSS version 25. Normally distributed variables were presented in mean ± SD and non-normal distributed variables were presented as median and interquartile range (IQR). In normal

distribution, ANOVA was used to test the significance in groups. Post hoc Tukey's test was applied for comparison between groups. In non-normal distribution Kruskal

Wallis H test was used for comparison among groups and Man whitney U test for comparison between groups.

Results

Leukocytosis was induced in all asthma induced groups. The treatment with *Cassia angustifolia* high dose, dexamethasone and emodin was successful in reducing leukocytosis to a statistically non-significant different values than normal control. *Cassia angustifolia* low and medium dose of ethanolic leaf extract were not successful at reducing elevated TLC. (Table I)

Eosinophilia was induced in all asthma induced groups. The treatment with *Cassia angustifolia* high dose, dexamethasone and emodin was successful in reducing eosinophilia to a statistically non-significant different values than normal control. *Cassia angustifolia* low and medium dose of ethanolic leaf extract were not successful at reducing elevated eosinophil levels. (Table I)

Values in all experimental groups were significantly lower than disease control group. While the dexamethasone, emodin, *Cassia angustifolia* medium and high dose treatment groups reduced IgE level to a non-significant difference from normal control group, the difference among *Cassia angustifolia* Low dose, medium dose and high dose were non-significant among themselves and from dexamethasone and emodin control groups.

While all the treatment groups had non-significantly different IL-13 level from normal control group, the difference between disease control and all the treatment groups was significant. *Cassia angustifolia* leaf ethanolic high dose treatment group had significantly lower IL-13 level than medium and low dose treated groups.

Discussion

Asthma is one of the quickly developing diseases. Asthma consists of variety of symptoms like wheezing, breathlessness, chest tightness, and cough.[12] Respiratory infections, molds, house dust are common asthma triggers.[13] The current asthma treatment is of high cost and has many side effects. Due to the drug-associated side effects of glucocorticoid, and specific skills needed for administration of inhalational drugs people with asthma are not getting full advantages.[14] Previous studies show that asthma control can be improved using natural products and these products are of low cost and show fewer side effects.[15] This study was conducted for evaluation of the anti-inflammatory effect of *Cassia angustifolia* on rat model of ovalbumin induced asthma in comparison to dexamethasone and emodin.

Serum TLC, DLC, IgE and IL-13 levels were measured at the termination of study**.** In this study TLC was significantly reduced than disease control group in high dose *Cassia angustifolia* group which was comparable to dexamethasone and emodin treated groups. The reduction in TLC was not significant in low dose and medium dose *Cassia angustifolia* treated groups.

In this study eosinophil count was raised in all asthma induced groups which indicates presence of disease in the experimental animals. Eosinophil count was significantly decreased in high dose *Cassia angustifolia* treated group, dexamethasone and emodin treated group. The reduction in low and medium dose *Cassia angustifolia* group was not significant

In this study the IgE antibodies were significantly reduced in all treatment groups including low and medium dose *Cassia angustifolia* treated groups. This decrease in IgE antibodies suggests that *Cassia angustifolia* possess anti-allergic properties and can modulate the immune response associated with allergic airway disease

In this study IL-13 was significantly reduced in all treated groups than disease control group. Though, the reduction was more significant in medium and high dose *Cassia angustifolia* treated groups. This reduction was statistically significant than low dose *Cassia angustifolia* treated group. This also shows the anti-inflammatory

Table I: TLC, eosinophil count, IgE antibody level and IL-13 level of ovalbumin asthma induced rats and rats treated with *Cassia angustifolia* leaves ethanolic extract, dexamethasone and emodin treated rats

Groups	TLC	Eosinophils	IgE level (IU/L)	IL-13 level (ng/L)
Normal Control	11.7500±1.66 x 10^3	3.0 ± 0.89 x 10^3	3.15 ± 0.82	2.33 ± 0.91
Diseased Control	17.80 ± 1.71 x 10^3	13.66 ± 2.06 x 10^3	15.65 ± 1.36	5.68 ± 1.22
Dexamethasone Control	9.93 ± 0.71 x 10^3	2.83 ± 0.75 x 10^3	2.83 ± 0.55	1.15 ± 0.91
Emodin Control	11.35 ± 1.44 x 10^3	3.0 ± 0.89 x 10^3	4.03 ± 1.02	1.38 ± 0.71
C. A. Low Dose	18.72 ± 1.39 x 10^3	14.50 ± 1.05 x 10^3	6.02 ± 1.78	2.43 ± 1.11
C.A. Medium Dose	16.50 ± 1.19 x 10^3	10.83 ± 1.47 x 10^3	5.10 ± 0.95	1.95 ± 0.58
C.A. High Dose	9.28 ± 0.69 x 10^3	2.83 ± 0.75 x 10^3	4.15 ± 0.67	1.91 ± 0.81
ANOVA	< 0.001	< 0.001	< 0.001	< 0.001

potential of *Cassia angustifolia*. It highlights the potential of *Cassia angustifolia* as a natural therapeutic agent for the management of allergic respiratory conditions by targeting IL-13-associated inflammatory processes.

Conclusion

Cassia angustifolia high dose extract has anti-inflammatory effects comparable to emodin and dexamethasone. However, it did not show superiority to emodin or dexamethasone. The dexamethasone was more successful in controlling chemical pathways of inflammation, IL-13 and IgE antibodies while the emodin, *Cassia angustifolia* were more successful in reducing the anti-inflammatory cells.

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