REPORT

Anti-stress and anti-allergic effect of *Actiniopteris radiata* in some aspects of asthma

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Abstract: Successive extracts of whole plant of *Actiniopteris radiata* screened for its therapeutic potential as an antiallergic and antistress agent in asthma using specific *in vivo* animal models. Only ethanol extract (AREE) at a higher dose of 100 mg/kg i.p significantly ($p<0.05$) decreased milk induced eosinophilia by 16.20 ± 2.235 when compared with control group while even lower doses of 50 mg/kg, i.p exhibited significant inhibition ($P<0.05$) of leukocytosis induced by milk in mice. Other extracts like petroleum ether, ethyl acetate and methanol unable to exhibit that significant potential. Results obtained thus validate the traditional claim of the *Actiniopteris radiata* utilization in different aspect of asthma due to presence of various polar secondary metabolites in ethanol extract.

Keywords: *Actiniopteris radiata*; leucocytosis, eosinophilia, anti-stress, anti-allergic, asthma.

INTRODUCTION

Asthma is a complex chronic inflammatory disease of airway with characteristics bronchospasm, increased mucous secretions and mucosal edema. Airway obstructions are episodic and reversible. It continues to be leading cause of mortality worldwide. Regardless many recent advances in modern medicines, the current modes of therapy do not offer the cure of the disease but control the symptoms with significant side effects. To avoid toxic side effects of modern medicine use of traditional drugs in the management of asthma related ailments is considered safe and more suitable. Herbal drugs grabbing more importance over chemotherapeutic agents because of their histo-compatability less toxic and fewer side effects. Herbal therapy provides rational means for the treatment of many internal diseases which are considered to be obstinate and incurable in other systems of medicine. Today is a need to search for natural drugs having utility in asthma-like conditions specifically towards mediators antagonists including antagonist of histamine, cytokines, PAF or inhibitors of prostaglandin synthesis and inhibition of key inflammatory cells like eosinophils, T lymphocytes along with stabilization of mast cell and thereby mediators release. Whole plant of *Actiniopteris radiata* (Sw.) Link. (Actiniopteridaceae) is a leptosporanginate fern, wildly distributed in overall India and tropical Africa at an altitude range of 600-1220 m. This plant is described in the Indigenous system of medicine for its utility as astringent, anti-inflammatory, useful in cough, bronchitis, asthma, diarrhea, dysentery, dysuria, used internally and externally for infected wounds and ulcers (Khare CP, 2004). Quercetin-3-rutinoside (Taneja, 1972), β-Sitosterol (Reddy, 2008) detected in different extracts of the plant. Plant known to posses antioxidant (Manjunath *et al*., 2009); antibacterial (Parihar *et al*., 2006); antifertility (Dixit, 1974) activity. Selected plant revered for its medicinal properties since ancient times. Regardless some investigation reported in literature more specific and scientific exploration thought to be much more essential to affirm the traditional claim as which was not yet done so far. Plant contains the polyphenolic compounds such as the flavonide, glycoside, alkaloid and sterol which are helpful in respiratory infections. Thus present work aimed to pharmacologically characterize the possible utility of selected plant as an antiallergic and adaptogenic in treatment of asthma.

MATERIALS AND METHODS

Chemicals

Chemicals and reagents like RPMI Buffer medium 1640, Eosin solution, WBC diluting fluid used was purchased from Hi Media and Qualigen.

Experimental animals

Healthy adult female Wistar rats weighing 200-250 g, albino Swiss mice weighing 25-30 g were used for the studies. They were housed in groups of five under standard laboratory conditions of temperature (25 ± 2°C) and 12/12 hr light/dark cycle. Animals had free access to standard pellet diet and water *ad libitum*. The protocol of

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this study was approved the Institutional Animals Ethics Committee, Vide No. NIB/IACE/09-10/83 dated 15-1-2010.

Preparation of extract solution

Whole plant of *Actiniopteris radiata* dried in air, coarsely powdered and subjected to successive solvent extraction in Soxhlet extractor in the order of increasing polarity of solvents like petroleum ether (60-80^{o}C), ethyl acetate, ethanol (95%), methanol. Vacuum dried all the extracts to yield PEE (5.44%), EAE (2.92%), EE (11.0%), ME (1.13%) respectively. Solutions of extract were prepared in 5 % Polyethylene glycol.

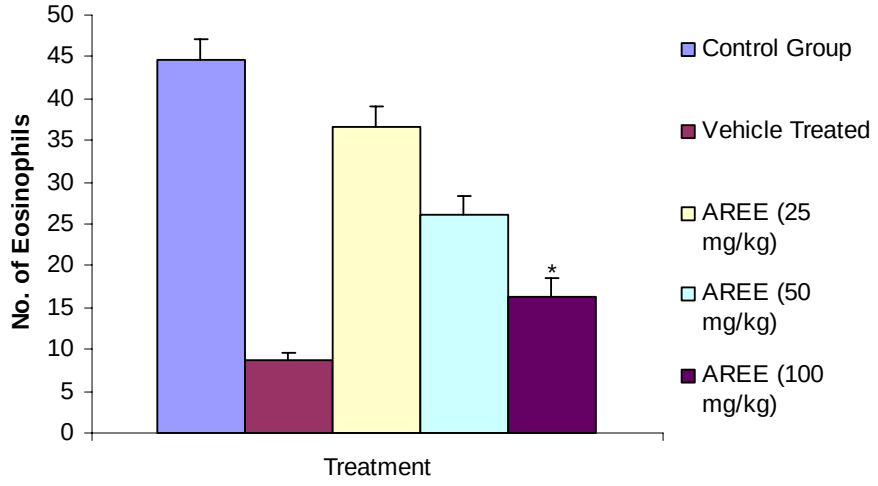

Fig. 1: Effect of AREE on Milk induced eosinophilia in mice
n=5, values are expressed in mean±SEM
*p< 0.05 compared with control group (ANOVA followed by Dunnett's test)
AREE- *Actinopteris radiata* ethanol extract

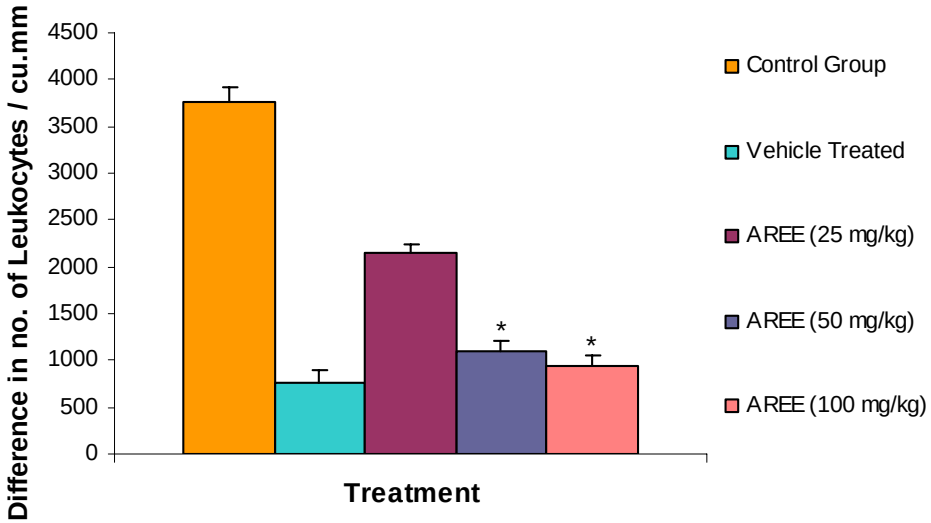

Fig. 2: Effect of AREE on Milk induced leukocytosis in mice
n=5, values are expressed in mean±SEM
*p< 0.05 compared with control group (ANOVA followed by Dunnett's test)
AREE – *Actinopteris radiata* ethanol extract

Acute toxicity study (LD_{50} determination) of different crude extracts

Organization for Economic Co-operation and Development (OECD) guideline 423 was used to determine the LD_{50} of various crude extracts as minimum number of animals are required. Review of literature reveals the prior studies of plant drug *in vivo* and so limit test was conducted at the highest starting dose level 2000 mg/kg of body weight.

In vivo assessment of antiallergic activity

Milk-induced eosinophilia in mice: Twenty five mice were divided into five groups of five animals each; Group I was served as control. Group II received only vehicle (5 % PEG-400, 1ml /kg, i.p.). Treatment group received test extracts at a dose of 25, 50,100 mg/kg, i.p.respectively, 1 hr before milk injection. Blood samples were withdrawn from retino-bulbar venous plexus with the help of a glass capillary under light anesthesia, Total eosinophil count was determined in each group before drug administration and 24 hr after milk administration. Blood was sucked in WBC pipette to mark 1, followed by the eosin solution to mark 11. Mixed the contents of the bulb thoroughly for 30-40 seconds and put it aside for 15-20 min for lysis and staining. Neubaur's chamber was charged with above fluid and eosinophil count was done.

In vivo assessment of antistress (adaptogenic activity)

Milk-induced leukocytosis in mice: Twenty five mice were divided into five groups of five animals each; Group I was served as control. Group II received only vehicle (5 % PEG-400, 1ml /kg, i.p.). Treatment group received test extracts at a dose of 25, 50,100 mg/kg, i.p. respectively, 1hr before milk injection. Blood samples were withdrawn from retino-bulbar venous plexus with the help of a glass capillary under light anesthesia. Total leukocyte count was done in each group before drug administration and 24 hr after milk injection.Blood was sucked in WBC pipette up to mark and further diluted with WBC diluting fluid. Pipette was shaken for few seconds and kept aside for five min. Neubaur's chamber was charged with above fluid and total leukocyte count was done. Difference in total leukocytes count before and 24 hr drug administration was calculated.

STATISTICAL ANALYSIS

The values were presented as mean±SEM. Statistical significance was calculated using one-way ANOVA followed by Dunnet comparison test. P values <0.05 were considered significant.

RESULT

Acute toxicity studies

Administration of single limit dose of 2000 mg/kg, p.o. of petroleum ether (60-80^{o}C), ethyl acetate, ethanol and methanol extracts of whole plants of *Actinopteris radiata* in female nulliparous rat emerged safe for further studies.

Effect of extracts on milk-induced eosinophilia in mice

Inflammatory role of eosinophill in late phase of asthma causes further infiltration of various mediators that promotes epithelial shedding, bronchoconstriction (Brigden, 1999; Taur *et al*., 2007). Blood eosinophilia usually seen with allergic respiratory disorder and that can be detectable on chest film (Ehright *et al*., 1989). Parental administration of milk produced a marked and significant increase in the counts of leukocytes/eosinophils 24hr after its administration (Bhargava, 1981). Brekhman and Dardymov also supported this theory (Brekhman *et al*., 1969). Our investigation reveals that vehicle treatment group reflects no eosinophilia while significant increase in total eosinophil count in vehicle and milk treated control group (44.60±2.541). Pretreatment with *Actiniopteris radiata* whole plant ethanol extract (AREE) at a highest dose of 100 mg/kg i.p. showed much significantly ($p<0.05$) decreased in eosinophilia (16.20±2.235) as compared to control group.

Effect of extracts on milk-induced leukocytosis in mice

The presence of antistress (adaptogenic) properties in some plant materials is being as described to be tonics in the Ayurvedic system of medicine (Selye, 1958). Mice pretreated with *Actiniopteris radiata* whole plant ethanol extract (AREE) at dose of 50 and 100 mg/kg, i.p. exhibited significant inhibition ($P<0.05$) of milk induced leukocytosis.

DISCUSSION

Actiniopteris radiata whole plant ethanol extract (AREE) potential to antagonize the milk induced blood eosinophilia as well as leucocytosis was confirmed and needs fractionation study of those therapeutic constituents. Present study revealed that AREE inhibits eosinopholia as well as leucocytosis count.

Finally it can be concluded that results thus obtained, validate the traditional claim of the *Actiniopteris radiata* (Sw.) Link. in utilization of different aspect of asthma.

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