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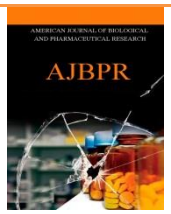

TO EVALUATE THE EFFECTS OF PHALERIA MACROCARPA ON AIRWAY INFLAMMATION AND AIR WAY REMODELING IN EXPERIMENTAL MODELS OF BRONCHIAL ASTHMA

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ABSTRACT

Asthma is an inflammatory disease of the airways that may result from exposure to allergens or other environmental irritants, resulting in bronchoconstriction, wheezing, and shortness of breath. Phaleria macrocarpa is a species of flowering plant in the family Thymelaeaceae. Extracts of P. macrocarpa have been used since years in traditional medicine that are evaluated scientifically as well. The extracts are reported for a number of valuable medicinal properties such as anti-cancer, anti-diabetic, anti-hyperlipidemic, anti-inflammatory, anti-bacterial, anti-fungal, anti-oxidant and vasorelaxant effect. The constituents isolated from different parts of P. macrocarpa include Phalerin, gallic acid, Icaricide C, magniferin, mahkoside A, dodecanoic acid, palmitic acid, des-acetylflavicordin-A, flavicordin-A, flavicordin-D, flavicordin-A glucoside, ethyl stearate, lignans, alkaloids and saponins. The present study was designed to investigate the immunomodulatory, antiinflammatory, and anti-airway remodeling effects of Phaleria Macrocarpa extract by assessing oxidative stress, immunological, and biochemical parameters in blood, BALF, as well as to evaluate the histopathological changes in lung tissue in experimental models of bronchial asthma in rats and to elucidate the possible mechanisms involved in these effects.

INTRODUCTION

Bronchial Asthma is a chronic inflammatory airway disease and is a major cause of morbidity and mortality worldwide. It remains a prevalent and challenging global health issue, with a tendency to consume valuable healthcare resources and impact human health. Several complexly interacting factors, external and internal, contribute to the pathophysiology and are potential risk factors for asthma. Chronic persistent inflammation in the airways can lead to airway remodeling (structural changes) which further complicates asthma management and enhances morbidity and mortality from the disease [1].

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The current conventional pharmacotherapy approach in asthma management includes antiinflammatory and bronchodilator agents, which are frequently given as combination therapy [2]. However, in view of the untoward effects and resultant compliance issues associated with such chronic pharmacotherapy, there is an unmet need and constant search for safer and viable alternative/complementary forms of therapy.

Medicinal plant-based agents have provided many important drugs for contemporary/modern medicine and are now rapidly emerging as alternative/complementary modes of therapy in chronic disorders including those of the respiratory tract[3]. *Phaleria Macrocarpa* is a well-documented medicinal herb effectively used in Indian traditional systems of medicine. The diverse pharmacological properties, viz., adaptogenic, anti-inflammatory, and immunomodulatory effects make it a

phytomedicine with immense potential for treating a wide range of chronic disorders. However, very few systematic studies have been conducted to explore the potential role of *Phaleria Macrocarpa* in the treatment of bronchial asthma.

Materials and Methods

Plant material

The Roots *Phaleria Macrocarpa* was collected from the herbal garden of Kanchipuram district of Tamilnadu during the month of January 2024. The plant material was identified & authenticated by botanist Dr. A.Mohan Raj and its Voucher specimen (No. PARC/2024/810) was preserved for future references.

Experimental Animals

Wistar albino rats ranging between 120-160 g of either sex were used. Rats were maintained in stainless steel cages under constant conditions of temperature (23 ± 2 ˚C), relative humidity (60 ± 2%) and lighting (12 h light / dark cycle).

The animals had free access to water *ad libitum* and fed with pellet diet (Lipton India Ltd., Mumbai, India.) except 1 h before and during the experiments. All experimental procedures were carried out in strict accordance with the guidelines prescribed by the Committee for the Purpose of Control and Supervision on Experimentation on Animals and were approved by the Institutional Animal Ethics Committee (IAEC) from Pallavan Pharmacy college, Kanchipuram (2308/PO/Re/S/2024/CCSEA).

Preparation of Extract

Collected parts of *Phaleria Macrocarpa* were cleaned with water and shade dried until a constant weight was obtained and subsequently powdered and sieved in mesh number 40. Powdered material (3 kg), i.e the marc was extracted with aqueous (100 % water solvent) at in Soxhlet apparatus (7 L) for 72 h. Dark brown semi-solid residue (598 g) was obtained by evaporating the aqueous extract under reduced pressure. The extract further used for pharmacological study.

Acute oral toxicity test (AOT)

AOT for *Phaleria Macrocarpa* Extract was performed in Wistar albino rats as per OECD guideline 425 (OECD, 2008). Animals were divided into 4 groups of 3 animals in each. Female, nulliparous and non-pregnant rats, 10 - 12 weeks and weighing between 110- 140 g, in addition, 12 - 16 weeks, male wistar rats, and weighing 110 - 190 g, were selected for this study. The animals were kept fasting overnight providing only with water, after which the PM extract were administered orally. The doses selected were 200 mg/kg, 400 mg/kg, and 2000 mg/kg. Another one group received distilled water as vehicle control. Their weight were taken and recorded before administration.

The dose at which mortality was observed in two out of three rats was considered as toxic dose. All the animals were observed twice daily for health condition, morbidity and mortality for 14 days. Based on the result obtained from this study, the dose for this study was fixed.

Experimental planning

To evaluate the anti-inflammatory and immunomodulatory effects of PM extract in an experimental model of bronchial asthma in rats by measuring:

a) Ovalbumin (OVA)-specific Ig E in blood and BALF
b) Tumor necrosis factor alpha (TNF-α) and associated cytokines (IL-4, IL-6) in blood and BALF
c) Histone deacetylase 2 (HDAC2) levels in blood and BALF[4]
d) Cytology of blood and BALF (absolute eosinophils count)

To study the effects of PM extract on oxidative and nitrosative stress in OVA-induced asthma and airway remodeling in rats:

a) Malondialdehyde (MDA) levels in blood and BALF: a stable marker of lipid peroxidation
b) Reduced glutathione (GSH) levels in blood and BALF, an antioxidant marker.
c) Nitric oxide (NOx) levels in blood and BALF

To evaluate the effects of PM extract on OVA-induced bronchial airway remodeling in rats by assessment:

a) IL-13 and IL-10 levels, in lung tissue and BALF.
b) TGF-β and hydroxyproline levels in lung tissue and BALF
c) Periostin levels in serum and BALF[5].
d) Lung histopathology.

Experimental Designing

Rats were randomly divided into 5 groups (n = 6/group) as follows:

(i) **Normal control:** rats were sensitized only with intra-peritoneal injections (i.p) of 0.5 ml normal saline (NS) and treated orally with distilled water for 14 days;
(ii) **Disease control** (OVA immunized/challenged group): rats were immunized and challenged with OVA and treated orally with distilled water;
(iii) OVA immunized/challenged group plus **PM extract (200 mg/kg, p.o.);**
(iv) OVA immunized/challenged group plus **PM extract (400 mg/kg, p.o.);** and (vi) Dexamethasone (positive control) group: rats were immunized and challenged with OVA and

treated with DEX (1 mg/kg, i.p.) from day 1 to day 14. In both groups (iii) and (iv), rats were immunized and challenged with OVA and treated orally with PM extract at the dose of 200 or 400 mg/kg from day 1 to day 14.

Immunization and challenge protocol

The animals were actively immunized and challenged with OVA. Briefly, on day 1, all rats, except normal controls, were immunized with OVA (10 mg per rat, i.p.) emulsified with 1 mg of AL(OH)3 as an adjuvant in 0.5 ml of NS. Two weeks after sensitization, on day 14, all rats, except the normal control group, were challenged with an i.p. injection of 1mg OVA in 0.5 ml of NS. In the treatment groups, oral administration of PM extract (200 or 400 mg/kg) or i.p. injection of dexamethasone was given 30 min before OVA exposure from day 1 to day 14. After 24 h of the challenge (on day 15), all animals were anesthetized with a combination of ketamine and xylazine (50/10 mg/kg i.p.).

Results & Discussion

Acute oral toxicity test (AOT)

As per observations and calculations from Acute Oral Toxicity (OECD Guidelines 425), the LD_{50} value of *Phaleria Macrocarpa* was found at 2000 mg/kg b.w., and therefore, 1/10th of this dose (200 mg/kg b.w.) was taken as the test dose for further study.

Pharmacological Evaluation of PM Extract Against Airway Inflammation

Effect of PM extract on OVA-specific IgE levels in blood and BALF

Table 1: Effect of Phaleria Macrocarpa extract (PM 200 and 400 mg/kg) on OVA-specific IgE levels in serum and BALF in OVA-immunized and challenged rats.

Group	OVA-specific IgE(foldchange)	
	Serum	BALF
NC	1.06±0.20	0.970±0.08
OVA	2.833±0.86*	1.89±0.15**
PM 200	1.333±0.17	1.19±0.23#
PM 400	1.121±0.16#	1.13±0.06#
DEX	0.923±0.15#	0.947±0.08#

Data are expressed as Mean ± S.E.M. (n = 6/group). *p < 0.05, **p < 0.01 vs normal control, #p< 0.05, ##p < 0.01 vs disease control group.

Effects of PM extract on oxidative and nitrosative stress mark ersin blood and BALF

Table 2 : Effect of Phaleria Macrocarpa extract (PM 200and400mg/kg) on OVA-induced changes in MDA levels in serum and BALF in OVA-immunized and challenged rats.

Group	MDA levels (µM/ml)	
	Serum	BALF
NC	4.527±1.05	3.09±1.11
OVA	18.26±5.97*	11.13±1.86**
PM 200	11.58±2.52	6.47±1.75
PM 400	5.61±1.73#	4.49±1.95#
DEX	4.32±1.11#	3.59±0.82#

All data are expressed as mean± S.E.M.(n=6).*p<0.05,p< 0.01vs normal control group,#p <0.05compared with disease control.**

Evaluation of PM extract against airway remodeling

Effect of PM extract on OVA-induced changes in cytokines levels in lung tissues and BALF

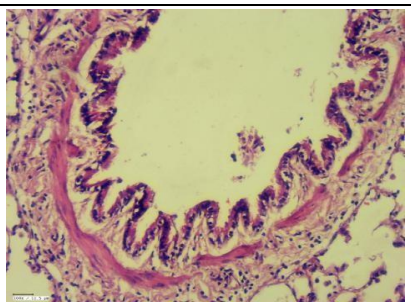
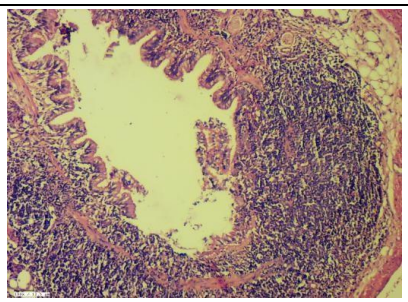
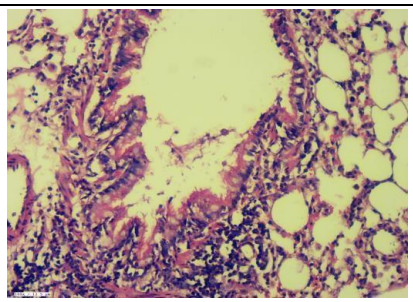
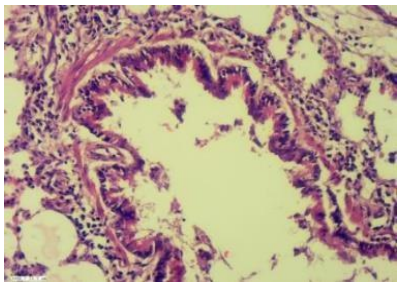
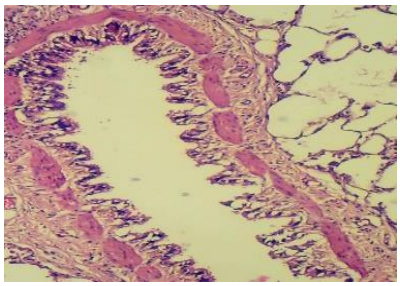
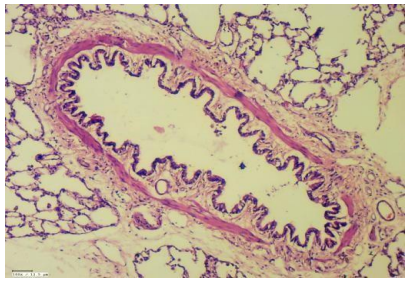
Table 3: Effect of Phaleria Macrocarpa extract on IL-13 levels in blood andBALFin OVA.

Group	IL-13levels(pg/ml)	
	Lung tissue	BALF
NC	15.28±1.37	86.48±4.44
OVA	26.56±3.65**	163.7±16.26**
PM 200	18.12±0.94#	129.6±10.74
PM 400	15.75±0.98##	116.3±9.63#

DEX	17.41±0.77#	100.7±8.14##
DEX+WS	16.83±1.15##	110.5±12.66#

All data are expressed as Mean ± SEM (n=6/group). **p < 0.01 vs NC, #p < 0.05, ##p <0.01 vs OVA sensitized rats.

Figure 1: Effects of Phaleria Macrocarpa extract (PM 200and400mg/kg) on OVA-induced changes in lung histology of rats. Hematoxylin and eosin (10x.), stained lung tissue.

Normal	OVA	OVA+PM200
		
OVA+PM 400	**OVA+DEX**	**OVA+DEX+PM**
		

DISCUSSION

The study results showed that the animal models of airway inflammation and airway remodeling were well established using OVA (immunization + challenge) model in rats and clearly showed a significant increase in the levels of inflammatory and fibrotic cytokines, as well as oxidative stress, in blood, BALF, and/or lung homogenate, which were significantly higher in OVA (disease control) group than in all other treatment groups6. In the experimental model of airway inflammation, immunization and challenge with OVA caused substantial increases in OVA-specific IgE, IL-4, and TNF-α levels, as well as eosinophil count in blood and BALF. OVA immunization and challenge significantly increased the levels of oxidative stress markers, i.e., MDA and NO. On the other hand, a marked reduction was observed in antiinflammatory, HDAC2, and anti-oxidant, GSH, levels in blood and BALF as compared to normal control rats. Pretreatment with PM extract (200 and 400 mg/kg, p.o., x 14 days) significantly attenuated OVA-induced eosinophil cell infiltration, IgE, IL-4, and TNF-α levels. In addition, treatment with WS extract significantly attenuated OVA-induced oxidative/nitrosative stress and restored GSH and HDAC2 to normal levels in blood and BALF in a dose-dependent manner [7]. These effects were comparable with those of THE standard (comparator) drug, dexamethasone. In the experimental model of airway remodeling, immunization and challenge of rats with aerosolized OVA significantly increased the levels of IL-13, TGF-β, hydroxyproline, and periostin in BALF, lung homogenate, and/or serum as compared to normal controls [8]. Further, OVA sensitization and challenge induced marked histopathological changes in the lungs, viz., characteristic of airway remodeling such as accumulation of intra alveolar fluid, goblet cell hyperplasia, increased alveolar septal thickness, sub-epithelial collagen deposition, and severeperibronchial inflammatory cell infiltrate. PM extract markedly downregulated IL-13, TGF-β, hydroxyproline, periostin, and, the oxidative DNA damage marker, levels in blood and BALF of asthmatic (OVA) rats. Pre-treatment with PM extract effectively attenuated the histopathological changes and maintained the structural integrity of the airways. The effects of PM on pulmonary pathology were more marked after treatment with the higher dose of plant extract (400 mg/kg), and these observations were

comparable with those seen in lung sections from DEX and combination treatment (DEX+PM) groups [9].

Airway obstruction was found to be strongly associated with airway inflammatory/immune responses, as evidenced by significant increases in Penh values, TNF- , IL-4, and OVA-specific IgE, as well as decreased IL-10 levels in the blood and BALF of OVA challenged rats (disease controls) compared to normal rats10. However, these changes in Penh values and immune markers were appreciably reversed after treatment with PM extract. The results were more marked at a higher dose of the extract (400 mg/kg) and were comparable to a great extent with those of dexamethasone.

CONCLUSION:

Taken together, the present data provide evidence that Phaleria Macrocarpa suppressed inflammatory cell infiltration and downregulated several inflammatory and pro-inflammatory cytokines in OVAinduced asthma in rat models. Moreover, PM could have potential therapeutic effects on airwayremodeling through inhibition of oxidative stress-induced DNA damage, modulation of inflammatory and fibrotic cytokines, and attenuation of the structural (histopathological) changes in the lungs that are associated with chronic persistent asthma and poor asthma control. The dose-dependent nature of the Phaleria Macrocarpa effects and its comparability with the standard drug, DEX, further highlights the pharmacological significance of this finding. The synergy between PM and DEX on various biomarkers and histopathological changes also indicates the possibility of herb-drug interactions and a complementary role of PM to standard treatment with corticosteroids or other drugs. Thus, Phaleria Macrocarpa could be considered a potential therapeutic agent and/or an effective adjunct to conventional drug therapy for asthma and other allergic conditions.

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