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Effectiveness of *Amorphophallus paeoniifolius* hydroalcoholic extract in asthma using network pharmacology and OVA–LPS rat model

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Amorphophallus paeoniifolius hydroalcoholic extract (HAE) possesses reported anti-inflammatory and bronchodilator properties and is traditionally used for asthma and cough. The present study aimed to investigate its anti-asthmatic potential using network pharmacology, followed by experimental validation. Major phytoconstituents of HAE were screened for target prediction and protein–protein interaction analysis. Gene ontology and KEGG pathway enrichment analyses were performed using STRING. Anti-asthmatic activity was evaluated in an ovalbumin–lipopolysaccharide (OVA–LPS)-induced rat model. Haematological parameters, inflammatory markers, Th2 cytokines, and lung histopathology were assessed and analysed using one-way ANOVA ($p < 0.05$). Lupeol, quercetin, resveratrol, stigmasterol, and β-sitosterol were identified as key bioactive compounds targeting asthma-related genes. Enrichment analysis highlighted TNF, Toll-like receptor, NOD-like receptor, and MAPK signalling pathways as major inflammatory regulators. *In vivo*, HAE significantly ($p < 0.05$) reduced total and differential leukocyte counts, Th2 cytokines, mast cell infiltration, airway inflammation, mucus hypersecretion, and bronchial hyperresponsiveness. Histopathology confirmed attenuation of inflammatory cell infiltration and airway remodelling. HAE exerts significant anti-asthmatic effects through multi-target immunomodulatory and anti-inflammatory mechanisms.

Keywords: *Amorphophallus paeoniifolius*, Asthma, Gene ontology, KEGG pathway, Network pharmacology, Th2 cytokines

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Introduction

Asthma is a major global health burden and affects individuals of all ages. Pathophysiologically, bronchial asthma involves airway inflammation, bronchoconstriction, mucosal oedema, eosinophilic infiltration, and airway hyperresponsiveness. Despite advances in therapy, the global prevalence of asthma continues to rise, particularly in developing countries. Corticosteroids are widely used in asthma management due to their potent anti-inflammatory and immunosuppressive effects. However, prolonged or high-dose corticosteroid therapy is also associated with significant adverse effects and necessitates careful monitoring. Therefore, there is a need to explore alternative or complementary therapeutic agents that are effective, safer, and capable of targeting multiple inflammatory pathways. Plants are considered a goldmine for their anti-inflammatory effect.

Amorphophallus paeoniifolius (Family: Araceae) is ethnomedicinally used for inflammatory disorders, respiratory ailments such as cough and asthma. Traditionally, the tuber has been used in Ayurvedic and folk medicine for the management of inflammatory disorders, gastrointestinal disturbances, piles, asthma, abdominal pain, dysentery, and metabolic diseases. Several pharmacological studies have demonstrated significant biological activities of *A. paeoniifolius*. Methanolic extracts of the tuber exhibited notable anti-inflammatory activity through modulation of inflammatory mediators. Owing to its rich phytochemical composition and therapeutic potential, the plant has attracted considerable scientific interest in recent years[1-3].

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Supplementary table is available online only.

Neuropharmacological investigations revealed synergistic depressant, anticonvulsant, and anxiolytic effects of *A. paeoniifolius* extracts in experimental animals[3–6]. Phytochemical investigations of *A. paeoniifolius* have revealed the presence of flavonoids, phenolic compounds, alkaloids, tannins, steroids, glycosides, carbohydrates, and bioactive secondary metabolites that contribute to its pharmacological activities[7-12]. These findings suggest possible central nervous system modulatory properties of the plant. Additionally, toxicity studies reported that standardised tuber extracts exhibited acceptable safety profiles at therapeutic doses. The leaves and tubers have also shown antidiarrheal and gastrokinetic activities in experimental animal models, supporting their traditional use in gastrointestinal disorders. Antioxidant, antimicrobial, and cytotoxic properties of *A. paeoniifolius* have been extensively investigated. Methanolic extracts exhibited antioxidant and cytotoxic effects against various cell lines, including breast cancer cells, where induction of apoptosis and inhibition of cell migration were observed. Antimicrobial activities against periodontal pathogens and other human pathogens have also been demonstrated using aqueous, ethanolic, and tuber extracts[13–16]. Beneficial effects in diabetic neuropathy and hyperlipidemia management have also been reported. These pharmacological effects may be attributed to the antioxidant and metabolic regulatory potential of the phytoconstituents present in the plant[17–19].

Furthermore, Analgesic activity has also been demonstrated in Swiss albino mice[20], beneficial effects against ulcerative colitis and constipation have been documented, indicating the plant's therapeutic potential in digestive system ailments. Recent research trends have focused on green synthesis and nanotechnology applications using *A. paeoniifolius* extracts. Plant-mediated synthesis of silver, gold, and zinc oxide nanoparticles has shown promising antibacterial and material science applications. Moreover, the plant extract has been explored as a potential green corrosion inhibitor and biopesticide, highlighting its industrial and environmental relevance. The plant has also shown promising antidiabetic and antihyperlipidemic activities in various experimental models. Studies in streptozotocin-induced diabetic rats and zebrafish models confirmed significant antihyperglycemic effects of tuber and leaf extracts[21–29].

Advanced phytochemical standardisation approaches using HPLC and HPTLC techniques have also been reported for quality evaluation of the plant extracts and biomarkers. In addition, flavonoid characterisation studies have further strengthened the understanding of the plant's bioactive profile. Computational approaches including molecular docking, ADMET prediction, and dynamic simulation studies, have further identified anti-inflammatory therapeutic targets associated with the bioactive constituents of the tuber extract[30–33].

Toxicity studies have shown that the hydroalcoholic extract is safe up to 2000 mg/kg in acute oral toxicity and up to 1000 mg/kg in subacute oral toxicity. Previous studies have demonstrated that, hydroalcoholic extract produces anti-inflammatory and immunomodulatory effects at doses of 200 mg/kg and 400 mg/kg. Moreover, *in-silico* investigations, including ADMET analysis and molecular docking, have suggested strong binding affinity of its phytoconstituents (Lupeol, Quercetin, Resveratrol, Stigmasterol, β-sitosterol and betulinic acid) toward key inflammatory targets such as TNF-α and MAPK3, which are critically involved in asthma pathogenesis. However, despite ethnobotanical claims and preliminary computational evidence, comprehensive mechanistic studies integrating network pharmacology with experimental validation in a relevant asthma model are lacking.

Network pharmacology provides a systematic approach to understand plant-based multi-component therapeutics. Protein-Protein Interaction (PPI) and Gene Ontology (GO) analysis are integral, core components of network pharmacology. Additionally, KEGG pathway enrichment analysis, a crucial component of network pharmacology identifies key mechanisms in asthma. They are used to identify disease-associated targets, construct complex interaction networks (drug-target-disease) by mapping molecular pathways and functional annotations, as well as explain significant enrichment in immune-related pathways, cell differentiation, cytokine-cytokine receptor interactions and MAPK signalling[34-36]. Confirmation of therapeutic efficacy of drug(s) or molecule(s) is done by experimental validation using animal studies. Ovalbumin-lipopolysaccharide (OVA–LPS) induced asthma in rat is most acceptable and relevant model to study anti-asthmatic property of drug(s) or molecule(s).

Despite extensive reports on the anti-inflammatory, antioxidant, antidiabetic, and antimicrobial activities of *A. paeoniifolius*, its therapeutic potential in asthma has not been thoroughly investigated. Previous studies mainly focused on general anti-inflammatory effects and preliminary *in silico* predictions, while detailed mechanistic evaluation in asthma-specific experimental models is lacking. Moreover, no study has integrated network pharmacology with *in vivo* validation using the OVA–LPS-induced asthma rat model to elucidate the anti-asthmatic potential and molecular mechanisms of hydroalcoholic extract of *A. paeoniifolius*. Therefore, the present study was designed to explore its effectiveness against asthma through network pharmacology and experimental validation using ovalbumin-lipopolysaccharide (OVA-LPS) induced asthma rat model.

Materials and Methods

Plant collection and extract preparation

In September month of 2024, tubers of *A. paeoniifolius* were collected and authenticated by Dr Kalpeshkumar Ishnava, Department of Biosciences, Bakrol, Anand and the specimen was stored under specimen number SPU-20241 at Department of Biosciences, Bakrol, Anand, for future references. The hydroalcoholic extract from tuber part was prepared by percolation method and further lyophilized which was used to check anti-asthmatic activity. Fig. 1 reflects plant of *A. paeoniifolius*.

Fig. 1 — Plant along with tuber part of *Amorphophallus paeoniifolius*.

Network pharmacology study

Identification of phyto molecules and their targets

The phytoconstituents from the plants *A. paeoniifolius* were retrieved from the Indian Medicinal Plants, Phytochemistry and Therapeutics 2.0 (IMPPAT 2.0; https://cb.imsc.res.in/imppat/) database. The targets modulated by the bioactives were sourced from the DIGEP-Pred 2.0 database (https://www.way2drug.com/digep-pred/) keeping pharmacological activity greater than 0.9. The targets involved in the pathogenesis of Asthma were retrieved from the Comparative Toxico-genomics Database (CTD; https://ctdbase.org/). The targets possessing an inference score greater than 90 were included for matching.

Network construction and Gene ontology analysis

The KEGG pathway and gene ontology enrichment analysis was performed using STRING (https://string-db.org/), and potential pathways involved in the pathogenesis of asthma were identified. Afterwards, network was constructed using Cytoscape for plant-bioactive-pathway-protein interaction network, treated as directed and analysed based on edge count, with node size and colour mapped accordingly. Moreover, advanced topological analysis was performed using node degree distribution and betweenness centrality by degree, incorporating parameters such as eccentricity, neighbourhood connectivity, in-degree distribution, and out-degree distribution to assess the structural properties, network robustness, target relevance and influence of each node within the network.

In-vivo experimental design

Ethics approval

The study protocol (RPCP/IAEC/2024-25/R1) was approved by institutional animal ethical committee (IAEC) for conducting experiment as per the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA).

Animals

Study protocol (RPCP/IAEC/2024-25/R1) for animal experimentation was reviewed and authenticated by institutional animal ethical committee (IAEC). Adult Wistar albino rats were procured from Zydus Research Centre (ZRC), Ahmedabad, Gujarat, India. Housing of animals and all experimentations were conducted as per CCSEA

(The Committee for Control and Supervision of Experiments on Animals) guidelines.

Preparation of drugs

Ovalbumin (OVA) suspension (100 mg/mL) was prepared by mixing with aluminium hydroxide gel. Lipopolysaccharide (LPS) was prepared in the concentration of 10 mg/mL in phosphate buffered saline (PBS). *A. paeoniifolius* Hydroalcoholic extract and Dexamethasone were prepared in phosphate buffered saline.

Study design and treatment

Total 30 Wistar Albino Rats were randomly divided into six groups (n = 6) and treatment was provided as per respective group. Group 1 served as the Normal Control (NC), in which rats were sensitised and challenged with normal saline. Group 2 served as the Disease Control (DC), where rats were sensitised and challenged with ovalbumin (OVA) and lipopolysaccharide (LPS). Group 3 received hydroalcoholic extract of *A. paeoniifolius* at a dose of 200 mg/kg (AP200) following OVA–LPS sensitization and challenge. Group 4 received the hydroalcoholic extract at a dose of 400 mg/kg (AP400) after OVA–LPS sensitization and challenge. Group 5 served as the standard control (Dexa), in which rats were sensitised and challenged with OVA–LPS and subsequently treated with dexamethasone at a dose of 3 mg/kg.

Disease induction model and Study protocol

In the Normal Control (NC) group, rats received intraperitoneal and aerosolised phosphate-buffered saline (PBS) for sensitisation and airway challenge, respectively.

In Groups 2, 3, 4, and 5, sensitisation was carried out on day 0 by intraperitoneal administration of ovalbumin (100 mg/kg) adsorbed onto aluminium hydroxide gel in PBS, along with lipopolysaccharide (LPS) in PBS. Subsequent airway challenges were performed on days 1, 3, 5, and 7. Pharmacological treatment was initiated on day 7 and continued through day 29. A last airway challenge was performed 18 hours before sacrificing the animals on 29th day. Sensitisation to animals was done by introducing them in aerosol of both allergens. On the 29th day, blood was collected in EDTA treated tube. Whole blood sample was used to estimate total and differential WBC, lymphocyte, Eosinophil count and granulocytes. Plasma was separated out using centrifugation at and BALF Mast stabilization, and inflammatory cytokines (TNF-α & Interleukin 4, 5 & 13) in Lung homogenate were measured. Histology of lungs was also performed.

Evaluation parameters

Blood collection and analysis

On day 29, after euthanasia, blood samples were collected on immediate basis and EDTA collection tube was used for collecting blood samples. Total and differential WBC were obtained using haematology analyser. From collected blood sample, plasma was separated and further used for estimation of TNF-α, IL-4, IL-5 and IL-13.

BALF collection and analysis

Bronchiolar lavage fluid (BALF) was collected by using Ryle's tube (number 5) into bronchi with flushing of 2 mL normal saline. Mast cells were collected by centrifuging BALF for 10 min at 4℃ at speed of 5000 rpm. These cells in the pellet were resuspended in 0.5 mL saline and Toluidine blue stain was used for staining purpose to observe under microscope. In BALF, total WBC and differential WBC were estimated.

Lung isolation and evaluations

Lungs were excised, washed, cleaned and divided in two parts. One part was used to evaluate cytokines level and second part was used to analyse histopathological changes.

Cytokines level

Lung was isolated and 10% (w/v) homogenate was prepared in phosphate-buffered saline using a tissue homogeniser at 3000 rpm for 5 minutes. Prepared homogenate was centrifugated at 5000 rpm for 10 min at 4℃ and supernatant was collected. Cytokines level (TNF-α, IL-4, IL-5 and IL-13) in collected supernatant were quantified through enzyme-linked immunosorbent assay (ELISA) using Rat TNF α kit, Rat IL-4 kit, Rat IL-5 kit, and Rat IL-13 kit in duplicate. Automated plate reader was used to analyse all plates.

Lung histopathology

Another portion of lung was fixed in 10% neutral buffered formalin solution for histopathological analysis. Tissues were processed and stained with haematoxylin and eosin (H&E). CX23 Biological Microscope (Olympus, Kyoto, Japan) was used

for examination and at 10× magnification, photomicrographs were captured.

Statistical analysis

All data were expressed as mean ± SD (standard deviation). Statistical analysis was performed using one-way ANOVA to assess differences among multiple groups using Microsoft Excel. $P < 0.05$ was considered statistically significant in all analyses. Tuckey's multiple comparison test was employed as post-hoc tests used after ANOVA.

Results

Network analysis

A total of 542 genes were identified to be modulated by *A. paeoniifolius*, of which 154 genes were associated with asthma pathogenesis. Such high overlap suggests a high probability of molecular interactions between *A. paeoniifolius* bioactive compounds and asthma-related biological processes.

A protein–protein interaction (PPI) network was constructed by mapping the target genes to the STRING database, resulting in a network comprising 153 nodes and 4632 edges. An average node degree of 60.5 was observed, suggesting that each protein interacted with over 60 other proteins on average, indicating a highly interconnected network architecture. The average local clustering coefficient was 0.722, indicates a high level of modularity and probability of forming functional clusters or complexes. Interestingly, the number of edges (4632) was greatly higher than expected number (1751) in an equivalent-sized random network, with a PPI enrichment p-value of $< 1.0e{-}16$, which is extremely significant. This indicates that proteins in the network are biologically connected and highly likely to share common signalling pathways or processes, which highlights functional significance of the discovered interactions (Fig. 2).

Pathway enrichment analysis of the 154 common targets of *A. paeoniifolius* and asthma, using the KEGG database, revealed the TNF signalling pathway (hsa04668) to be the most significantly enriched, with 30 of 111 genes involved (FDR = 2.74E-33). The pathway contains key regulators like TNF, IL6, MAPK1/3/8/9/14, and NFKB1, all of which are the key mediators of airway inflammation and remodelling in asthma. Additionally, Toll-like receptor signalling pathway (hsa04620) and NOD-like receptor signalling pathway (hsa04621), both critical to innate immune response and inflammatory signalling, were significantly enriched (FDR = 2.34E-24 and 6.65E-20, respectively). These pathways activate downstream NF-κB and MAPK cascades resulting in cytokine release and immigration of immune cells into the airway. The Th17 (hsa04659), Th1 and Th2 cell differentiation (hsa04658), and T cell receptor signalling (hsa04660) pathways indicate the importance of adaptive immune cell polarisation in asthma. Notably, dysregulation of Th2 and Th17 responses is closely linked with allergic inflammation and severe asthma phenotypes. The MAPK signalling pathway (hsa04010), with 26 genes enriched, further indicates the importance of signal transduction networks in regulating cytokine production, epithelial cell apoptosis, and airway hyperreactivity. Additionally, pathways like NF-κB signalling (hsa04064), cytokine–cytokine receptor interaction (hsa04060), and chemokine signalling (hsa04062) were enriched, confirming their central role in inflammatory cascades and the chemotactic immigration of immune cells into the lungs. The asthma-specific KEGG pathway (hsa05310) was enriched directly (FDR = 1.10E-05), involving genes like IL4, TNF, IL10, CD40, and CCL11. However, the relatively lower number of involved genes compared to upstream inflammatory pathways suggests that the therapeutic efficacies of *A. paeoniifolius* are likely to act through broader immunomodulatory and signalling pathways (Table 1). The significantly enriched KEGG pathways and their corresponding target proteins are summarised in Supplementary Table 1.

Network analysis of major bioactive phytomolecules like lupeol, quercetin, resveratrol, beta-sitosterol and stigmasterol-illustrates their ability to modulate pro-inflammatory signalling pathways. Lupeol and quercetin are highly connected, with degrees of 94 and 93, and betweenness centralities of 0.38 and 0.32 and closeness centralities of approximately 0.42-0.43, reflecting their key positions in primary network clusters. Resveratrol, with degree of 72 and betweenness centrality of 0.20, reflects a key position in the network. Beta-sitosterol and stigmasterol, with lower degrees of 61 and 43, reflect higher neighbourhood connectivity values (3.51 and 4.02) and radiality scores (0.68 and 0.65), reflecting their ability to interact with many proteins and modulate network communication. Stress values are diverse; lupeol and quercetin reflect high stress

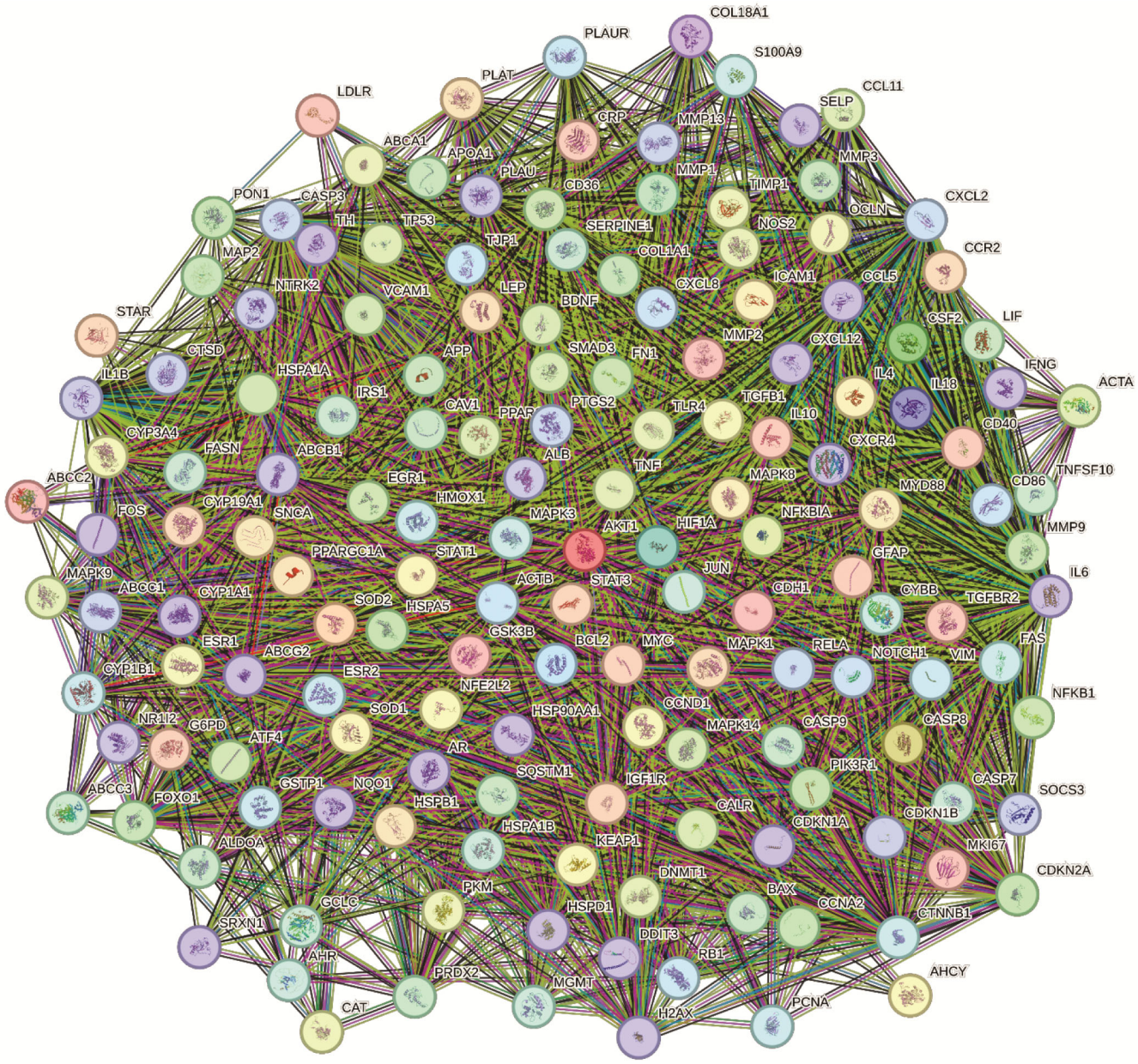

Fig. 2 — Result of specific protein-protein interaction of targets modulated via *Amorphophallus paeoniifolius* against asthma.

(851,300 and 971,098), reflecting their inclusion in many shortest paths within the network, whereas stigmasterol's lower stress (402,368), together with the highest topological coefficient (TC = 0.38), reflects a more specialised but powerful role (Table 2).

MAPK1 was recognised as a central node, with the highest degree value and stress value, and was thus identified as playing a central role in modulating inflammatory and stress responses through its involvement in the MAPK, TNF, and Toll-like receptor signalling pathways. Similarly, RELA and NFKB1, the core subunits of the NF-κB transcription complex, were also identified as important inflammatory mediators that facilitate the expression of important cytokines such as IL-6, TNF-α, and IL-1β, all of which play important roles in asthma pathogenesis. JUN, another member of the AP-1 complex, also plays an important role in T-cell differentiation and cytokine regulation and thus impacts the immune dysregulation characteristic of asthma. Another MAP kinase, MAPK3, has also been

Table 1 — The disease-pathway association for the enriched KEGG pathways

Category	Pathway Name	KEGG ID	Role in Asthma
Core	Asthma	hsa05310	Represents central molecular mechanisms of asthma including immune dysfunction.
Immune Response	Th1 and Th2 cell differentiation	hsa04658	Th2 response is dominant in allergic asthma; Th1/Th17 modulate inflammation.
	Th17 cell differentiation	hsa04659	Associated with severe asthma and neutrophilic inflammation.
	T cell receptor signalling	hsa04660	Activates Th cell-mediated allergic responses.
	B cell receptor signalling	hsa04662	Essential for IgE production and memory B cell activation.
	Toll-like receptor signalling	hsa04620	Detects allergens/pathogens, triggers innate immune responses.
	NOD-like receptor signalling	hsa04621	Drives inflammasome activation and epithelial inflammation.
	Antigen processing and presentation	hsa04612	Initiates adaptive immunity via MHC-mediated allergen presentation.
Cytokine Signalling	Cytokine-cytokine receptor interaction	hsa04060	Involves IL-4, IL-5, IL-13—critical cytokines in allergic asthma.
	Chemokine signalling pathway	hsa04062	Mediates migration of immune cells to inflamed airways.
	TNF signalling pathway	hsa04668	Promotes airway inflammation and remodeling.
Structural Remodelling	Focal adhesion	hsa04510	Linked to airway smooth muscle migration, fibrosis, and stiffness.
	Tight junction	hsa04530	Barrier dysfunction allows allergen and microbe penetration.
Signal Transduction	MAPK signalling pathway	hsa04010	Regulates inflammatory gene expression and cell survival.
	NF-kappa B signalling pathway	hsa04064	Controls transcription of pro-inflammatory mediators and cytokines.

Table 2 — The parameters obtained upon network analysis for *Amorphophallus paeoniifolius*

Name	ASPL	BC	CC	Degree	NC	Radiality	Stress	TC
Lupeol	2.34	0.38	0.43	94	2.47	0.78	851300	0.08
Quercetin	2.37	0.32	0.42	93	2.89	0.77	971098	0.10
Resveratrol	2.54	0.20	0.39	72	2.97	0.74	615510	0.12
Beta-sitosterol	2.92	0.12	0.34	61	3.51	0.68	944644	0.28
Stigmasterol	3.09	0.04	0.32	43	4.02	0.65	402368	0.38
TNF signalling pathway (hsa04668)	2.87	0.07	0.35	30	5.47	0.69	181558	0.24
MAPK signalling pathway (hsa04010)	2.89	0.07	0.35	26	5.23	0.68	168204	0.22
NOD-like receptor signalling pathway (hsa04621)	2.91	0.04	0.34	23	6.43	0.68	126384	0.29
Toll-like receptor signalling pathway (hsa04620)	2.91	0.03	0.34	23	6.91	0.68	129128	0.31
Cytokine-cytokine receptor interaction (hsa04060)	3.38	0.04	0.30	22	3.68	0.60	139864	0.18
MAPK1	2.14	0.33	0.47	15	35.00	0.81	990174	0.13
RELA	3.58	0.00	0.28	10	19.90	0.57	10246	0.30
JUN	3.42	0.01	0.29	10	19.00	0.60	29586	0.26
MAPK3	3.55	0.00	0.28	10	19.90	0.58	11522	0.28
NFKB1	3.58	0.00	0.28	10	19.90	0.57	10246	0.30

*ASPL: Average Shortest Path Length; BC: Betweenness Centrality; CC: Closeness Centrality; NC: Neighbourhood Connectivity; TC: Topological Coefficient

reported to play a role in epithelial cell signalling and airway remodelling. These targets are common to multiple asthma-related KEGG pathways such as TNF signalling, Th17 cell differentiation, and Toll-like receptor signalling. Other targets such as IL4, IL6, TNF, CXCL8, CD40, TLR4, and PIK3R1 further point toward the involvement of AP bioactives in the modulation of important inflammatory and immune pathways.

TNF-alpha, IL-4, IL-5, and IL-13 are pivotal cytokines responsible for driving asthma pathogenesis through an integrated network of inflammation, airway hyperresponsiveness, and remodelling. TNF-alpha, a pro-inflammatory cytokine, activates the TNF signalling pathway (hsa04668), initiating NF-κB and MAPK cascades that enhance adhesion molecule (ICAM1, VCAM1) and chemokine expression, initiating airway inflammation and immune cell

recruitment. IL-4 and IL-13, hallmark Th2 cytokines, are pivotal for asthma (hsa05310) through triggering IgE class switch, mucus hypersecretion, and airway remodelling, acting through the Th1 and Th2 cell differentiation pathway (hsa04658) and cytokine-cytokine receptor interaction pathway (hsa04060), primarily through STAT6 signalling. IL-5 promotes eosinophil growth and survival, inducing eosinophilic inflammation hallmark of asthma. These cytokines collectively orchestrate through overlapping pathways e.g., NF-kappa B signalling pathway (hsa04064), Toll-like receptor signalling (hsa04620), and MAPK signalling (hsa04010), exacerbating airway inflammation and remodelling pathways underlying asthma's clinical manifestations (Fig. 3).

Gene Ontology analysis

Gene Ontology (GO) enrichment analysis was conducted using the STRING database covering three domains: Cellular Component (CC), Molecular Function (MF), and Biological Process (BP). GO analysis identified 113 CC in which Extracellular space (GO:0005615) scored the lowest false discovery rate of 8.93E-18 *via* the modulation of 76 observed genes *i.e.* HMOX1, AHCY, TIMP1, MMP2, PLAT, TGFB1, PON1, SERPINE1, ACTA2,

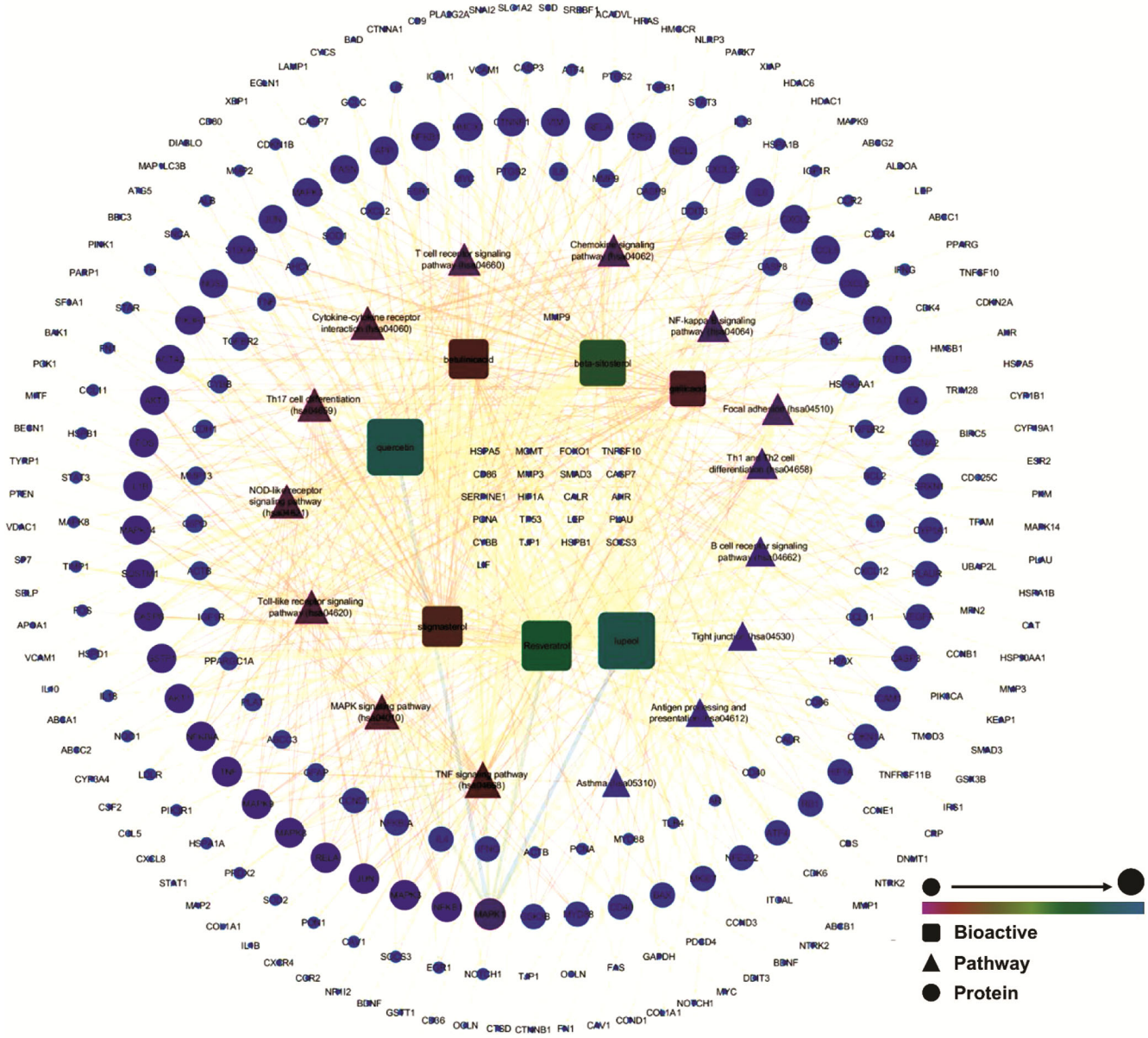

Fig. 3 — Result of bioactive-protein-pathway interaction for the targets modulated via *Amorphophallus paeoniifolius* against asthma.

COL1A1, IFNG, IL4, CTSD, APOA1, CAT, TNFSF10, HSPB1, LIF, CRP, MMP13, CDH1, IL1B, SELP, ICAM1, SOD1, IL18, APP, BAX, VCAM1, ALB, CSF2, MMP3, PRDX2, CCL11, FASN, CXCL8, LEP, PKM, CALR, HSPA5, CD86, HSP90AA1, HSPD1, FN1, TGFBR2, COL18A1, S100A9, CD40, MMP9, PLAU, HSPA1B, HSPA1A, PCNA, SQSTM1, G6PD, CXCL12, GSTP1, ABCC1, IL6, CXCR4, TNF, IL10, BDNF, CD36, CXCL2, H2AX, VIM, SOD2, LDLR, CCL5, ABCB1, ACTB, CTNNB1, ALDOA, FAS, SNCA against 3247 background genes at a strength of 0.48. Similarly, 179 MF were identified in which protein binding (GO:*0005515*) scored the lowest false discovery rate of 8.29E-35 *via* the modulation of 134 observed genes *i.e.* MAPK1, HMOX1, NFKBIA, AHCY, TIMP1, MMP2, PLAT, TGFB1, PON1, SERPINE1, ACTA2, COL1A1, NFKB1, CCND1, CDKN1B, IFNG, MAPK14, IL4, APOA1, EGR1, CAT, TNFSF10, AHR, HSPB1, LIF, GFAP, CRP, CDH1, MAPK3, IL1B, SELP, STAT3, ICAM1, PPARGC1A, RB1, TP53, SOD1, NTRK2, IL18, APP, PPARG, CCR2, BAX, VCAM1, ALB, CSF2, CCL11, FASN, IRS1, FOS, CXCL8, CASP3, LEP, NQO1, PKM, CALR, HSPA5, GSK3B, NOS2, CASP9, SOCS3, SMAD3, HSP90AA1, NR1I2, ATF4, CAV1, PLAUR, HSPD1, ESR2, FN1, OCLN, TJP1, CASP8, TGFBR2, COL18A1, MAP2, STAT1, PTGS2, MKI67, S100A9, JUN, CD40, MMP9, TLR4, AR, ABCA1, HSPA1B, HSPA1A, CYBB, PCNA, FOXO1, TH, SQSTM1, G6PD, KEAP1, CYP1A1, MAPK8, CXCL12, NFE2L2, BCL2, GSTP1, RELA, CDKN1A, IL6, CXCR4, TNF, ESR1, IL10, BDNF, CD36, CDKN2A, CXCL2, PIK3R1, H2AX, HIF1A, VIM, SOD2, DDIT3, AKT1, LDLR, CCL5, ABCB1, MYC, CCNA2, ACTB, CTNNB1, ALDOA, IGF1R, ABCG2, MYD88, FAS, NOTCH1, CYP3A4, SNCA against 7242 background genes at a strength of 0.38. Moreover, 2127 BP were identified where, Cellular response to chemical stimulus (GO:0070887) scored the lowest false discovery rate of 1.47E-73 *via* the modulation of 123 observed genes *i.e.* MAPK1, HMOX1, NFKBIA, MMP2, PLAT, TGFB1, SERPINE1, COL1A1, NFKB1, CCND1, CDKN1B, IFNG, MAPK14, EGR1, CAT, AHR, HSPB1, CDH1, MAPK3, IL1B, STAT3, ICAM1, PPARGC1A, RB1, TP53, SOD1, NTRK2, IL18, APP, ABCC3, PPARG, CCR2, BAX, VCAM1, ALB, CSF2, MMP3, PRDX2, MGMT, CCL11, FASN, IRS1, FOS, CXCL8, CASP3, LEP, NQO1, PKM, CALR, HSPA5, GSK3B, NOS2, CASP9, SOCS3, CD86, SMAD3, NR1I2, ATF4, CAV1, HSPD1, ESR2, TJP1, CASP8, TGFBR2, DNMT1, STAT1, PTGS2, S100A9, CASP7, JUN, CD40, MMP9, TLR4, AR, ABCA1, HSPA1B, HSPA1A, CYBB, PCNA, FOXO1, TH, SRXN1, G6PD, KEAP1, CYP1A1, MAPK8, CXCL12, NFE2L2, BCL2, GSTP1, ABCC1, RELA, IL6, CXCR4, MAPK9, TNF, ESR1, IL10, BDNF, CD36, CXCL2, PIK3R1, HIF1A, VIM, SOD2, DDIT3, AKT1, LDLR, CCL5, CYP1B1, MYC, CCNA2, ACTB, CTNNB1, IGF1R, ABCC2, GCLC, ABCG2, MYD88, FAS, NOTCH1, CYP3A4, SNCA against 2609 background gene count with a strength of 2.75 (Fig. 4).

***In-vivo* study along with statistical evaluation**

OVA-LPS rat model of asthma was used in which the evaluated parameters data showed greater efficacy of hydroalcoholic extract of plant. AP-200 and AP-400 showed great decrease in total WBC, differential WBC (Fig. 5 and Fig. 6), NLR ratio and mast cell stabilisation (Fig. 7), TNF-α (Fig. 8), along with IL-4, IL-5, IL-13 (Fig. 9). Histopathological evaluation is shown in Fig. 10, showing good activity of hydroalcoholic extract.

In normal control group, intact lung architecture with well-defined alveolar spaces, thin interalveolar septa, and absence of inflammatory cell infiltration, indicating normal pulmonary histology. In disease control group, marked pathological alterations are evident, including dense inflammatory cell infiltration, thickened alveolar septa, congestion and partial obliteration of air spaces, indicating severe airway inflammation and tissue injury. In standard control group, lung architecture appears substantially restored with reduced inflammatory infiltration, decreased septal thickening, and improved airway lumen, demonstrating significant anti-inflammatory effect of dexamethasone. Hydroalcoholic extract at 200 mg/kg dose showed moderate improvement is observed, with reduced inflammatory cell infiltration and partial restoration of alveolar structure, though mild septal thickening and residual inflammation persist. Hydroalcoholic extract at 400 mg/kg dose showed near-normal lung architecture with minimal inflammatory infiltration, well-preserved alveolar spaces and reduced septal thickening, suggesting a dose-dependent protective effect.

One-way ANOVA revealed significant differences among groups for all evaluated haematological

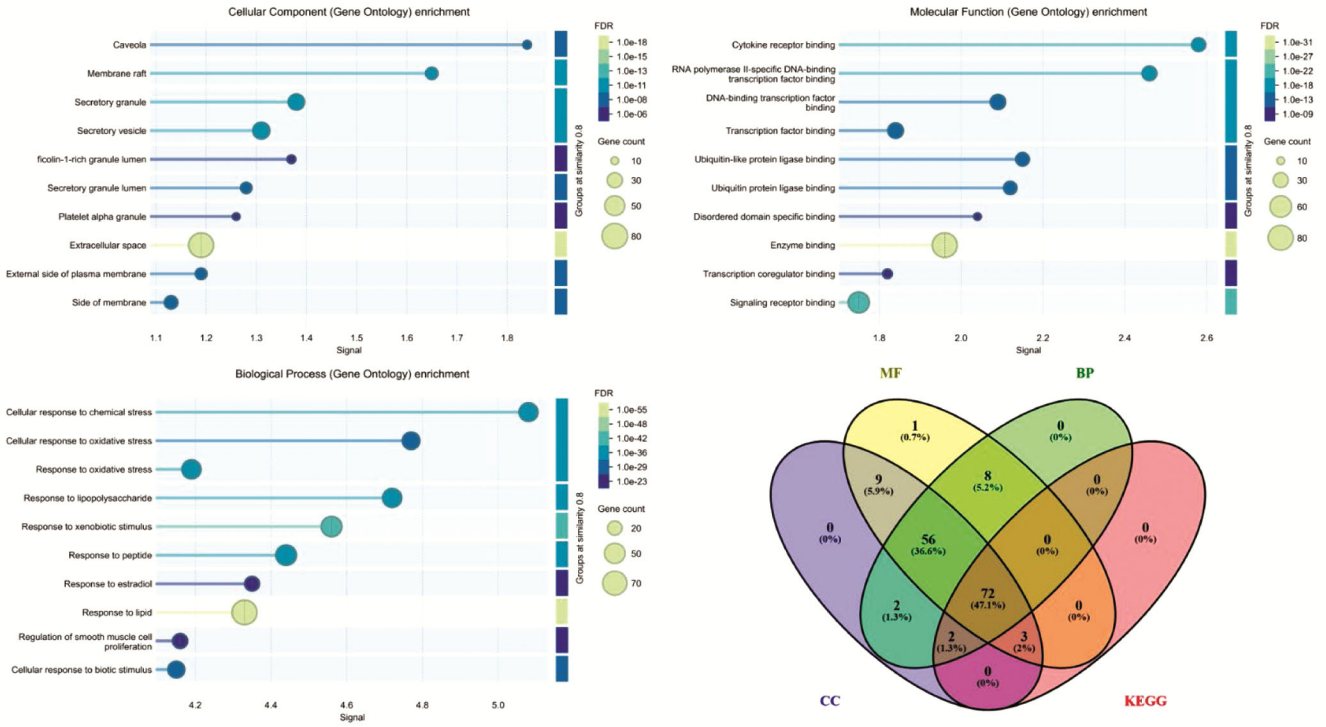

Fig. 4 — Result of enriched gene ontology for the top 10 cellular components, molecular function and biological processes modulated via *Amorphophallus paeoniifolius* against asthma and the common targets between KEGG enriched genes and gene ontology.

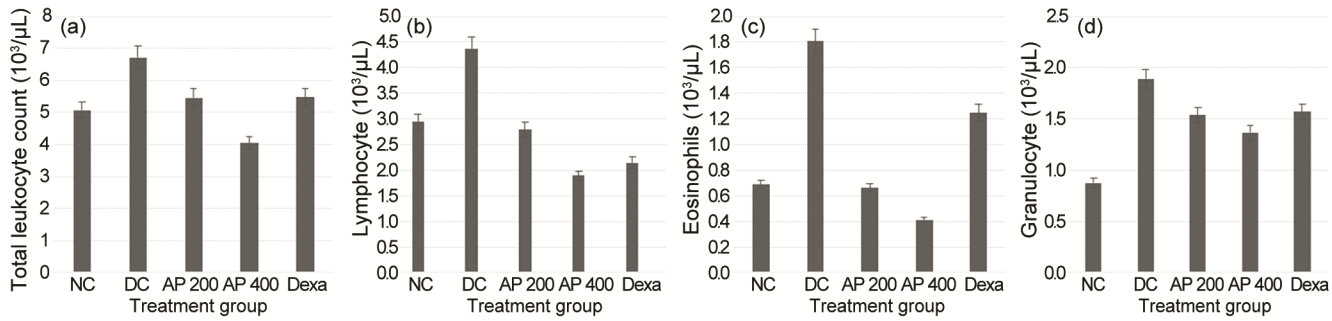

Fig. 5 — Effect of *Amorphophallus paeoniifolius* hydroalcoholic extract on hematological parameters (a) Total leukocyte count, (b) Lymphocyte, (c) Eosinophils, and (d) Granulocyte) in OVA–LPS-induced asthmatic rats.

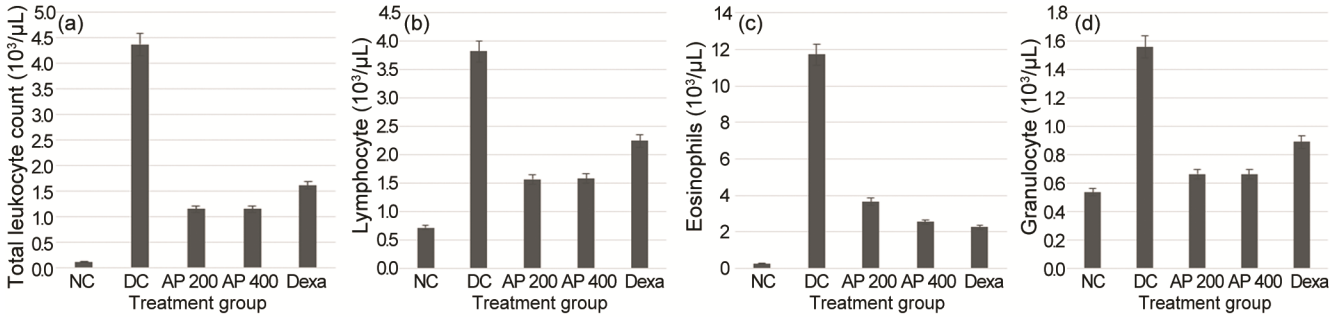

Fig. 6 — Effect of *Amorphophallus paeoniifolius* hydroalcoholic extract on bronchoalveolar lavage fluid (BALF) (a) Total leukocyte count, (b) Lymphocyte, (c) Eosinophils, and (d) Granulocyte) cellular profile in OVA–LPS-induced asthmatic rats.

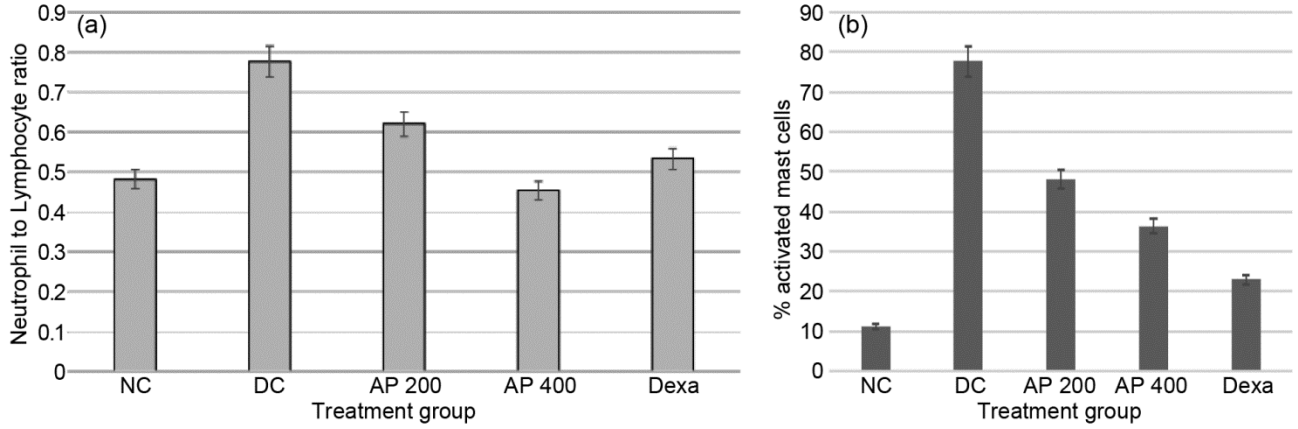

Fig. 7 — Effect of *Amorphophallus paeoniifolius* hydroalcoholic extract on (a) neutrophil-to-lymphocyte ratio (NLR), and (b) activated mast cells in BALF in OVA–LPS-induced asthmatic rats.

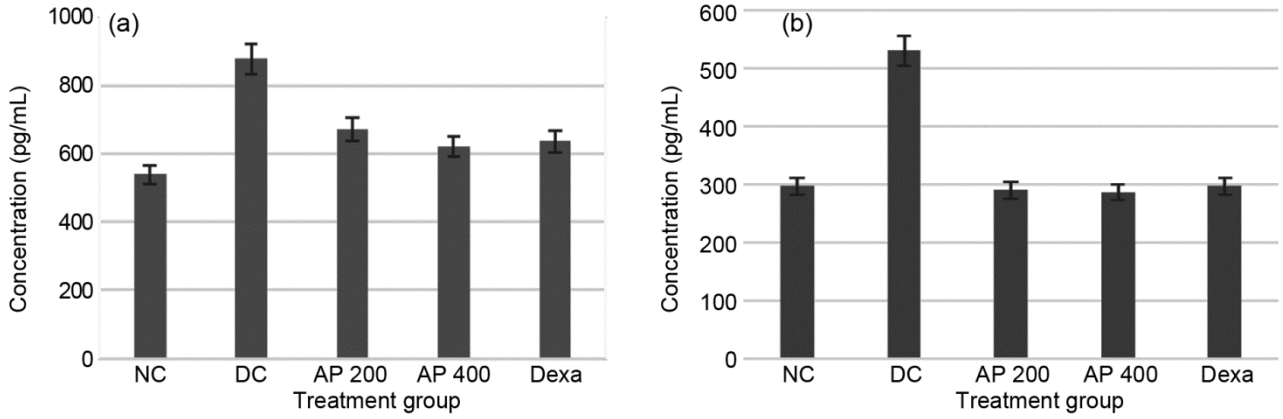

Fig. 8 — Effect of *Amorphophallus paeoniifolius* hydroalcoholic extract on TNF-α level in (a) lung homogenate, and (b) plasma in OVA–LPS-induced asthmatic rats.

parameters. Total leukocyte count showed a significant overall difference ($p = 0.003$). Tukey's post hoc analysis indicated a highly significant reduction in Total leukocyte count in the AP400-treated group compared with the disease control (DC) group ($p = 0.0005$), while NC also differed significantly from DC ($p = 0.0447$). Lymphocyte count demonstrated a highly significant overall variation ($p = 0.00019$), with significant differences observed between DC and AP200 ($p = 0.0204$), DC and AP400 ($p = 0.0002$), and NC and DC ($p = 0.0405$). Eosinophil also differed significantly among groups ($p = 0.003$), with highly significant reductions in AP200 and AP400 compared to DC ($p < 0.0001$), and a significant difference between NC and DC ($p < 0.0001$). Granulocyte count showed a significant overall difference ($p = 0.011$), with NC differing significantly from DC ($p = 0.0054$). Overall, treatment groups demonstrated marked improvement compared to disease control.

Analysis of bronchoalveolar lavage fluid (BALF) revealed significant differences among the experimental groups for all evaluated parameters. Total leukocyte count in BALF showed a highly significant overall difference by one-way ANOVA ($p = 1.03 \times 10^{-11}$). Tukey's post hoc test indicated significant differences between NC and DC ($p < 0.0001$), as well as between DC and the treatment groups AP200 ($p < 0.0001$) and AP400 ($p < 0.0001$). Lymphocyte count also showed a significant overall variation ($p = 0.0004$), with significant reductions observed in AP200 ($p = 0.0077$) and AP400 ($p = 0.0081$) compared to DC, while NC differed highly significantly from DC ($p = 0.0002$). Eosinophil count demonstrated a significant difference among groups ($p = 0.009$), with significant differences between DC and AP400 ($p = 0.0425$) and between NC and DC ($p = 0.0074$). Granulocyte count also showed significant variation ($p = 0.006$), with significant differences between DC and AP200 ($p = 0.022$),

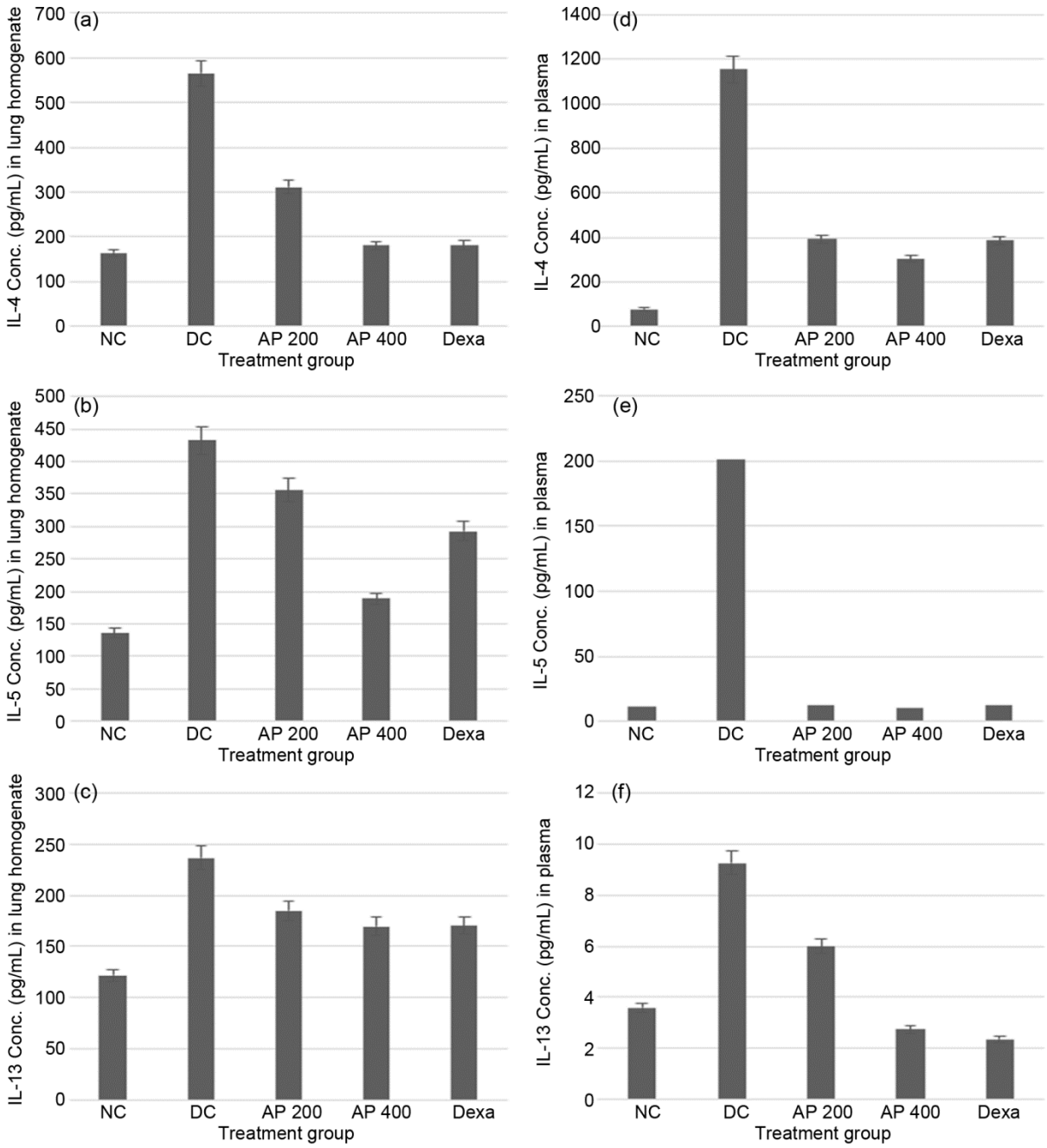

Fig. 9 — Effect of *Amorphophallus paeoniifolius* hydroalcoholic extract on IL-4, IL-5, and IL-13 level in lung homogenate and plasma in OVA–LPS-induced asthmatic rats.

DC and AP400 ($p = 0.021$), and NC and DC ($p = 0.007$), indicating improvement in treated groups.

One-way ANOVA revealed a significant difference among groups in the NLR evaluated ($p = 0.004$). Tukey's post hoc analysis demonstrated significant differences between DC and AP400 ($p = 0.0050$), DC and Dexa ($p = 0.0484$), and NC and DC ($p = 0.0115$). These findings indicate that both AP400 and dexamethasone treatment significantly improved the altered parameter compared with the disease control group.

For the mast cell stabilisation, ANOVA showed a highly significant overall difference among groups ($p = 0.00001$). Post hoc multiple comparison analysis revealed significant differences between NC and DC, DC and AP200, DC and AP400, and DC and Dexa. Additionally, a significant difference was observed between Dexa and AP400. These results suggest that

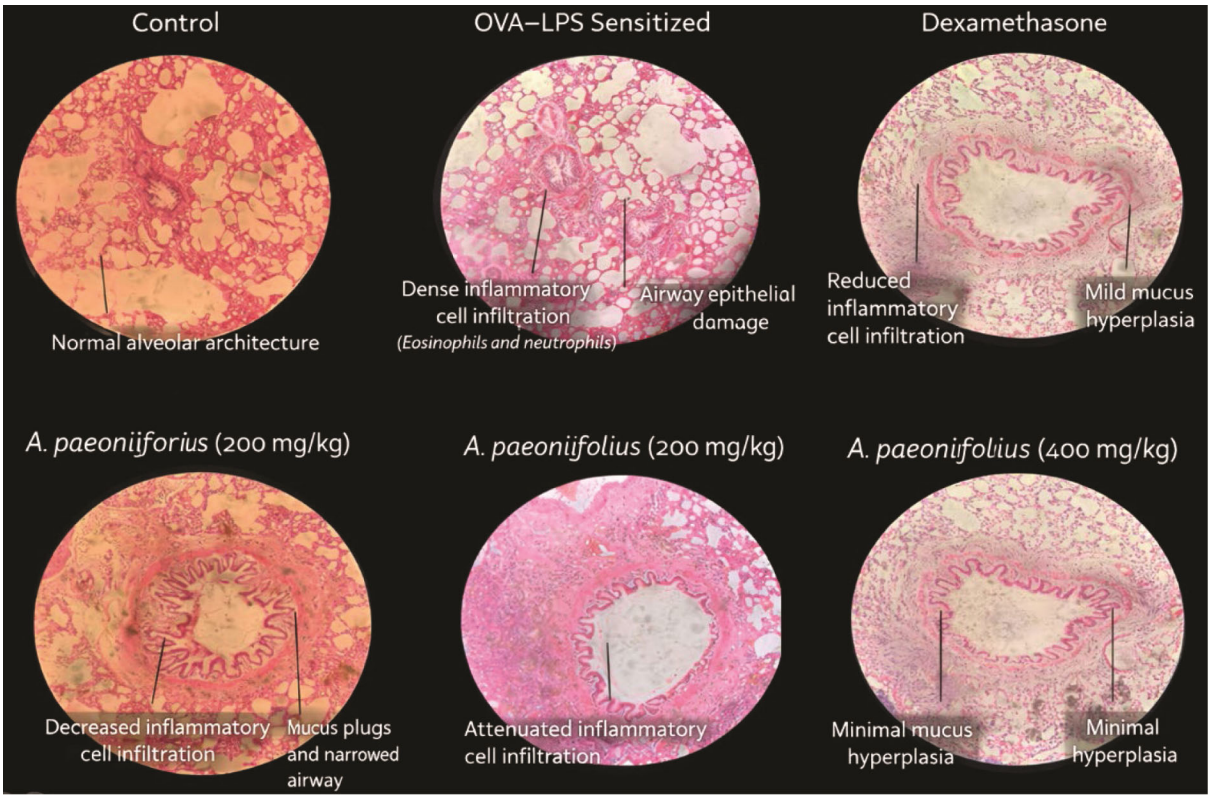

Fig. 10 — Effect of *Amorphophallus paeoniifolius* hydroalcoholic extract on histopathology study of lung in OVA–LPS-induced asthmatic rats.

both extract-treated groups and the standard drug markedly attenuated disease-associated alterations, with a differential effect noted between dexamethasone and the higher extract dose. Overall, the findings confirm substantial therapeutic modulation in treatment groups compared to disease control.

One-way ANOVA demonstrated significant differences among groups for most inflammatory cytokines assessed in lung homogenate and plasma. TNF-α levels in lung homogenate showed a significant overall difference ($p = 0.01$). Tukey's post hoc analysis revealed significant reductions in AP200 ($p = 0.0039$), AP400 ($p = 0.0003$), and Dexa ($p = 0.0006$) compared with DC, while NC also differed significantly from DC ($p < 0.0001$). Similarly, plasma TNF-α levels showed a highly significant overall difference ($p = 1.16 \times 10^{-5}$), with significant reductions in all treatment groups compared to DC.

IL-4 levels in lung homogenate ($p = 0.0012$) and plasma ($p = 0.017$) showed significant differences, with AP400 and Dexa markedly reducing cytokine levels compared with DC. IL-5 levels were also significantly altered in lung homogenate ($p = 0.002$) and plasma ($p = 0.0007$), with treatment groups demonstrating significant attenuation compared to DC. IL-13 levels in lung homogenate showed a significant overall difference ($p = 0.0013$), particularly in AP200 and AP400 groups versus DC. However, plasma IL-13 levels did not show a significant difference among groups ($p = 0.5$). Overall, treatment groups significantly suppressed pro-inflammatory and Th2 cytokines compared to disease control.

Discussion

The findings of the present study are consistent with previously reported network pharmacology-based investigations exploring herbal therapies for asthma. Network Pharmacology Jiaying *et al.* demonstrated that ChuanKeZhi Injection exerted significant anti-asthmatic and anti-inflammatory effects through regulation of multiple inflammatory mediators and signaling pathways associated with airway inflammation[34]. Similarly, Baolan *et al.* reported that Zao Ren An Shen Capsule exhibited therapeutic potential in asthma by targeting inflammation-related pathways and immune

modulation mechanisms identified through network pharmacology analysis[35]. Furthermore, Mingzhe *et al.* revealed that Wenyang Huayin decoction alleviated bronchial asthma through modulation of autophagy-related targets and pathways, highlighting the importance of multi-target mechanisms in herbal anti-asthmatic therapy[36].

In agreement with these studies, the present investigation demonstrated that hydroalcoholic extract of *A. paeoniifolius* may exert anti-asthmatic activity through multi-component and multi-target interactions associated with inflammatory and immune-regulatory pathways. The integration of network pharmacology with OVA-LPS induced experimental validation further supports the therapeutic relevance of plant-based interventions in asthma management and strengthens the translational significance of the current findings.

Network and pathway analysis revealed that *A. paeoniifolius* targets a substantial number of asthma-related genes, suggesting significant therapeutic potential. Among 542 genes modulated by the plant, 154 were associated with asthma, forming a highly interconnected protein–protein interaction (PPI) network consisting of 153 nodes and 4632 edges. This dense connectivity, highlighted by a high average node degree and local clustering coefficient, indicates robust functional relationships among the proteins. The PPI network's extremely significant enrichment ($p<1.0e{-}16$) implies that these proteins are not only structurally connected but are functionally co-regulated within key signalling pathways.

KEGG pathway enrichment analysis highlighted TNF, Toll-like receptor, NOD-like receptor and MAPK signalling pathways as key regulatory mechanisms underlying inflammation and immune responses in asthma. Bioactive compounds like lupeol, quercetin, and resveratrol emerged as central nodes in this network, exhibiting strong topological importance and capacity to influence multiple signalling hubs.

Gene Ontology (GO) enrichment analysis further supported the involvement of *A. paeoniifolius* in modulating inflammatory and immune-related cellular functions. Within the Cellular Component category, "extracellular space" showed the highest enrichment, consistent with the secretion of cytokines and signalling mediators involved in asthma pathophysiology. Molecular Function analysis highlighted "protein binding" as a predominant activity, indicating the high interaction potential of target proteins. Biological Process enrichment revealed "cellular response to chemical stimulus" as the most significant, underlining the responsiveness of these targets to inflammatory signals.

Importantly, key asthma-related cytokines such as TNF-α, IL-4, IL-5, and IL-13 were involved in multiple enriched pathways including NF-κB, MAPK, and T-cell differentiation pathways. These findings indicate that *A. paeoniifolius* modulates a broad spectrum of immune-regulatory mechanisms through its bioactive compounds acting on central network nodes and inflammatory pathways, highlighting its potential as a broad-spectrum immunomodulatory agent in asthma therapy.

Significant increase in leukocyte count observed in the disease control group suggests successful induction of asthma-like conditions and immune system activation. The elevation of lymphocytes, eosinophils, and granulocytes in the disease control group reflects enhanced immune activation and allergic inflammatory response characteristic of asthma. Compared with the normal control group, ovalbumin–lipopolysaccharide exposure significantly increased total and differential WBC counts ($p <0.05$) in model control group, whereas treatment with hydroalcoholic extract significantly ($p <0.05$) reduced these parameters, demonstrating its anti-inflammatory effect.

Asthma induction also resulted in significant inflammation characterised by increased neutrophil infiltration and a relative decrease in lymphocytes, which was observed in disease control. The Normal Control group had lowest NLR, reflecting a healthy baseline with minimal inflammation. AP-200 and AP-400 groups showed a dose-dependent reduction in NLR, indicating attenuation of systemic inflammation. The Dexa (Dexamethasone-treated) group also demonstrated a reduced NLR compared to the DC group, confirming its expected anti-inflammatory effects. However, the NLR reduction in the AP 400 group appears comparable or even more effective than Dexamethasone, highlighting its potential as a promising therapeutic agent ($p <0.05$).

Compared with the Normal Control (NC) group, ovalbumin–lipopolysaccharide (OVA–LPS) exposure significantly increased the number of degranulated mast cells ($p <0.05$). Treatment with the hydroalcoholic extract markedly attenuated

OVA–LPS–induced mast cell degranulation in BALF ($p < 0.05$), indicating its protective effect against mast cell activation and degranulation.

Furthermore, concentrations of pro-inflammatory cytokines TNF-α, IL-4, IL-5, and IL-13 were significantly reduced following treatment with the hydroalcoholic extract, demonstrating its pronounced inhibitory effect on key inflammatory mediators involved in asthma pathogenesis. The observed reduction in inflammatory cells, mast cell degranulation, and cytokine levels is consistent with the network pharmacology predictions, which indicated modulation of key inflammatory pathways such as NF-κB and MAPK signalling.

Normal Control displayed well-organised alveolar spaces, minimal inflammatory infiltration, and intact bronchial epithelium, indicating healthy lung architecture. Disease Control (LPS-OVA treated) showed severe airway inflammation, epithelial thickening, goblet cell hyperplasia and increased peri bronchial inflammatory infiltration, reflecting an asthmatic phenotype. Dexamethasone treatment exhibited reduced inflammation, preserved airway structure, and decreased mucus secretion, indicating effective suppression of asthma-related pathology. AP-200 treatment demonstrated partial reduction in inflammation and airway remodelling, with mild epithelial restoration and moderate immune cell infiltration. AP-400 treatment showed a greater reduction in inflammatory infiltration and epithelial thickening, suggesting better therapeutic efficacy compared to AP-200.

Overall, the combined network pharmacology and experimental findings suggest that *A. paeoniifolius* exerts protective effects against asthma through multi-target modulation of inflammatory pathways, supporting its potential as a promising therapeutic candidate.

Conclusion

Network pharmacology analysis revealed that *A. paeoniifolius* targets key asthma-associated genes and inflammatory pathways, particularly NF-κB and MAPK signalling. *A. paeoniifolius* demonstrated significant anti-asthmatic potential through a multi-target and multi-pathway mechanism. Experimental validation in OVA–LPS model confirmed these predictions, demonstrating reduced inflammatory cell infiltration, mast cell degranulation and pro-inflammatory cytokines (TNF-α, IL-4, IL-5, and IL-13), along with improved lung histology. Overall, these findings suggest that, hydroalcoholic extract of *A. paeoniifolius* exerts anti-asthmatic effects through multi-target modulation of immune and inflammatory pathways, supporting its potential as a promising therapeutic candidate.

Conflict of interest

The authors declare that there is no conflict of interest.

AI use disclosure

The authors declare that no artificial intelligence (AI)-assisted technology was used in the preparation, writing, analysis, or editing of this manuscript.

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