Research article

Supplementation of syringic acid-rich *Phrynium pubinerve* leaves imparts protection against allergic inflammatory responses by downregulating iNOS, COX-2, and NF-κB expressions

Md Arman Islam [a], Md Samiul Huq Atanu [b], Md Afjalus Siraj [a,b], Rabindra Nath Acharyya [a], Khondoker Shahin Ahmed [c], Shrabanti Dev [a,*], Shaikh Jamal Uddin [a], Asish Kumar Das [a]

[a] *Pharmacy Discipline, Life Science School, Khulna University, Khulna 9208, Bangladesh*
[b] *Department of Pharmacy, Gono Bishwabidyalay, Savar, Dhaka 1344, Bangladesh*
[c] *Chemical Research Division, BCSIR Laboratories, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka 1205, Bangladesh*

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ABSTRACT

Background: The present study was designed to characterize the role of ethanolic leaf extract of *Phrynium pubinerve* Blume (EPP) supplement in attenuating allergic inflammation, encouraged by the presence of syringic acid in it, as this phenolic acid is reportedly promising in suppressing serum immunoglobulin E (IgE) and inflammatory cytokine levels.

Materials and methods: HPLC-DAD dereplication analysis was performed to determine the presence of the vital polyphenolic metabolites. The efficacy of EPP against lipopolysaccharide (LPS)-induced inflammation in RAW 264.7 cells was evaluated by measuring its inhibitory effects on NO and ROS/RNS production. The expressions of major inflammation-associated molecules (iNOS, COX-2, NF-κB, IL-6, and TNF-α) in RAW 264.7 cells were assessed through Western blot. Physiological and behavioral changes, BMI, and different biochemical parameters in mice blood serum were investigated in the toxicological assays. Formaldehyde-induced paw edema test in mice was conducted using established animal model. TDI-induced allergic model in mice was carried out to determine different allergy-like symptoms, and differential white blood cell (WBC) counts in blood and bronchoalveolar lavage (BAL) fluid. The intermolecular interaction analysis of the identified major metabolite of EPP with H1R and iNOS was studied by molecular docking.

Results: HPLC-DAD analysis showed the presence of syringic acid (89.19 mg/100 g EPP) and a few other compounds. LPS-induced NO generation was reduced by EPP in a concentration-dependent manner, showing IC_{50} of 28.20 ± 0.27 µg/mL. EPP exhibited a similar inhibitory effect on ROS/RNS production with IC_{50} of 29.47 ± 2.19 µg/mL. Western blotting revealed that EPP significantly downregulated the expressions of iNOS, COX-2, NF-κB, IL-6, and TNF-α in RAW 264.7 cells when challenged with LPS. The toxicological assays confirmed the dosage and organ-specific safety of EPP. In the formaldehyde-induced paw edema test, EPP caused a 66.41% reduction in mice paw volume at 500 mg/kg dose. It ameliorated TDI-induced allergy-like symptoms and decreased different inflammatory WBCs in mice's blood and BAL fluid in a dose-dependent manner. Finally, syringic acid demonstrated mentionable intermolecular binding affinity towards H1R (−6.6 Kcal/moL) and iNOS (−6.7 Kcal/moL).

* Corresponding author.
  E-mail address: dshraban@yahoo.com (S. Dev).

Conclusions: Collectively, considerable scientific reasoning was obtained in favor of the suppressive potential of EPP against allergic inflammatory responses that are proposed to be exerted via the downregulation of iNOS, COX-2, and NF-κB expressions, H1R antagonism and suppression of cytokines, such as IL-6, and TNF-α.

1. Introduction

Allergic reaction, a highly patient-specific condition is characterized as a symptomatic response to a normally harmless environmental antigen. The clinical expression of the most common allergic diseases includes asthma, rhinitis, conjunctivitis, anaphylaxis, drug-, food and insect allergy, eczema, angioedema, etc. [1]. It is mediated by the extension of the T helper 2 (T_H2) subset of T cells, combined with isotype switching of B cells to produce immunoglobulin E (IgE) antibodies that are discrete for common environmental allergens [2]. The allergic response towards particular antigens demands a prior event called sensitization that constitutes a plethora of incidents which cause the IgE generation and its subsequent binding to high-affinity Fcε receptors on mast cells and basophils in the tissue [3]. Toluene 2, 4-diisocyanate (TDI) is a well-known chemical allergen, and if inhaled, it promotes inflammatory allergic rhinitis and asthma by aggravating the levels of IgE, and inflammatory cells, like eosinophils and cytokines, such as interleukin-4 (IL-4), IL-5, IL-13, and Interferon-gamma (IFN-γ) [4]. After second sensitization, IgE recognizes the foreign antigens, like TDI that in turn cause some primary and secondary events, where the primary event involves IgE-dependent mast cell degranulation and consequent release of histamine and heparin. Specifically, histamine is responsible for making the local environment difficult for antigens by enhancing mucus secretion and promoting sensory nerve stimulation accompanied by induction of scratching, sneezing, and coughing [5]. Histamine plays a cornerstone role in developing the allergic-associated inflammatory diseases, including asthma, allergic rhino-conjunctivitis, etc. via modulating the activation and maturation of white blood cells (WBCs) and their migration to target sites. In addition, it also exhibits different other immune-modulatory roles by regulating the actions of T and B cells, macrophages, neutrophils, eosinophils, monocytes, and dendritic cells [6]. Among the histamine receptors, both histamine H1 receptor (H1R) and H4R have mentionable contributions to the initiation and progression of histamine-associated allergic disorders. H1R, being significantly implicated in allergy and inflammation, is actively involved in smooth muscle contraction of the respiratory tract, augmentation of vascular permeability, and generation of prostacyclin and platelet-activating factor [7]. Therefore, instant hypersensitivity reactions, including edema, erythema, and pruritus are exerted by the activation of H1R [8]. As dysregulation of various inflammatory cells, including lymphocytes, neutrophils, eosinophils, etc. are often associated with several allergic conditions like asthma, disruption in the nasal airway, skin sensitivity, etc., inflammatory signaling proteins are expected to be closely implicated in allergic inflammation [9, 10]. Enhanced cyclooxygenase-2 (COX-2) expression plays a vital role in the pathobiology of allergy-associated respiratory diseases exemplified by dysregulated inflammation [11]. According to sufficient evidence, inflammatory signaling molecules, such as inducible nitric oxide synthase (iNOS) and nuclear factor kappa B (NF-κB) are also mechanistically involved in allergies [12,13].

Inflammation is a closely associated pathological condition with allergy which is a reflective feedback system of the body, mainly promoted through the invasion of harmful chemicals, pathogenic attack, or tissue damage due to external stress [14]. Induction of inflammation may emerge from endogenous, such as malfunctioned or damaged tissues, and exogenous origins, such as allergens, pathogens, reactive oxygen species (ROS), etc. Mast cells and macrophages in the tissues identify the infection and cause an immediate release of chemokines, ROS, and cytokines, including IL-1, IL-6, IFN-γ, *tumor necrosis factor α* (TNF-α), etc. [15]. iNOS is an important enzyme known for utilizing oxygen to produce nitric oxide (NO) free radicals which regulate various biochemical signaling pathways and energy metabolism, i.e., lipid and glucose metabolism under inflammatory conditions [16]. On the other hand, iNOS expression is highly dependent on the activation of NF-κB [17].

The treatment strategies for both allergy and inflammation are identical in many instances, as the pathophysiologic signaling mechanisms are interrelated: some common cytokines, such as COX-2 and iNOS are upregulated during cellular response against both [18]. As ROS is actively involved in both of these conditions, antioxidative treatment options are prevalent for both allergic inflammations, i.e., asthma, and inflammation alone, i.e., bowel disease [18]. The drugs available for treating allergic inflammation during allergic rhinitis primarily focus on the symptomatic relief. Furthermore, it is challenging to develop a single drug to tackle allergic inflammation, and most times it is hindered because of the complex disease pathophysiology [19]. Thus, the search for better treatment options for allergic inflammation is still relevant. Moreover, dietary supplements containing both allergy and inflammation suppressive properties can be handy for managing chronic diseases, including asthma, rheumatoid arthritis, osteoarthritis, etc. [20, 21].

Natural polyphenolic compounds are widely established as alternatives for managing various diseases [22–24]. Phenolic acids have caught much interest of late as they are an abundant source of antioxidants [15]. Hence, diseases predominantly caused by oxidative stress, i.e., allergy and inflammation can be effectively managed with agents rich in phenolic acids [25]. Hill tract plant *Phrynium pubinerve* Blume, belonging to the Marantaceae family is used as a traditional remedy in Xishuangbanna, China to relieve coughs, fever, and stomach disorders [26]. One of the species belonging to the same genus, *P. imbricatum* is traditionally used to treat cough, asthma, and catarrh by the Chakma ethnic community of Chittagong hill tract, Bangladesh [27]. Therefore, it is evident that the plants from the Phrynium genus are traditionally proven source that heal allergic conditions. Despite its promising use in the traditional healing practice, the Phrynium genus and especially, *P. pubinerve* is poorly studied. Moreover, the antioxidant potentials of *P. pubinerve* leaves have been demonstrated by different redox assays in the previously published literature [26]. Because of its strong antioxidant value, *P. pubinerve* (also called *P. capitatum*) leaves are used a food packaging material in Meghalaya, India [28]. Based on these backgrounds,

the present study is outlined to search for the role of *P. pubinerve* leaves against allergic inflammation.

2. Materials and methods

2.1. Chemicals and reagents

The analytical solvents and reagents utilized in this study, including dimethyl sulfoxide (DMSO), *fetal bovine serum* (*FBS*), Dulbecco's Modified Eagle Medium (DMEM), lipopolysaccharide (LPS), trichloroacetic acid (TCA), tris base, Griess reagent, sulforhodamine B (SRB) and radio-immunoprecipitation assay (RIPA) buffer were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Primary antibodies, such as iNOS (#13120), COX-2 (#12282), NF-κB (#8242), IL-6 (#12153), TNF-α (#6945), and β-actin (#3700) were bought from Cell Signaling Technology (Danvers, MA, USA). TDI was obtained from Wako Chemical (Tokyo, Japan). Tween-80, sodium nitrate ($NaNO_3$), ethyl acetate ($C_4H_8O_2$), acetic acid (CH_3COOH), HPLC grade methanol (MeOH), acetonitrile (CH_3CN), and water (H_2O) were of analytical grade and obtained from Merck (Darmstadt, Germany). Standard drugs cetirizine and ibuprofen were purchased from Square Pharmaceuticals Ltd. (Dhaka, Bangladesh).

2.2. Extraction and sample preparation

Phrynium pubinerve leaves were obtained from Chittagong, Bangladesh, and recognized by the botanical scientists of Bangladesh National Herbarium (Mirpur, Dhaka, Bangladesh) (Accession No. DACB 46532). The leaves were adequately cleaned with fresh water, shade dried for 4 weeks, and then ground with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). 500 g powder was soaked into 2000 mL 96% ethanol into a sealed glass container and kept for 14 days accompanied by occasional shaking and stirring to obtain optimum maceration. Ethanol was used for extraction because one of the previous studies on *P. pubinerve* used the same solvent and obtained sufficient extract [26]. The whole mixture was coarse filtered followed by another filtration using Whatman filter paper. The evaporated filtrate gave 12 g of greenish gummy mass (EPP; 2.4% yield). The solvent evaporation procedure was conducted couple of rounds to confirm the absolute minimization of the solvent presence.

2.3. Quantification of polyphenolic metabolites using HPLC-DAD

HPLC-DAD analysis of EPP was conducted [29] using Shimadzu (LC-20A, Japan) designed with a binary solvent delivery pump (LC-20AT), an auto sampler (SIL-20 HT), column oven (CTO-20A), and a photodiode array detector (SPD-M20A) regulated by the LC solution software (Lab solution separation was performed using Luna C_{18} (5 μm) phenomenex column (4.6 × 250 mm) at 33 °C. The mobile phase composed of A (1% acetic acid in acetonitrile) and B (1% acetic acid in water) with gradient elution: 0.01–20 min (5–25% A), 20–30 min (25–40% A), 30–35 min (40–60% A), 35–40 min (60-30% A), 40–45 min (30–5% A), and 45–50 min (5% A) was used in this study. The sample injection volume was 20 μL, and the flow rate was adjusted at 0.5 mL/min. The UV detector was fixed at 270 nm wavelength and applied to validate the method and analysis. The mobile phase was filtered through a 0.45 μm Nylon 6, 6 membrane filter (India) and degassed under vacuum. In order to prepare a calibration curve, a standard stock solution was formulated in methanol containing gallic acid (20 μg/mL); 3,4-dihydroxybenzoic acid (15 μg/mL); catechin hydrate (50 μg/mL); catechol, (−) epicatechin, rosmarinic acid (30 μg/mL each); caffeic acid, vanillic acid, syringic acid, rutin hydrate, *p*-coumaric acid, *trans*-ferulic acid, quercetin (10 μg/mL each); myricetin, kaempferol (8 μg/mL each); *trans*-cinnamic acid (4 μg/mL). Then, EPP was dissolved into methanol to prepare a solution of 10 mg/mL concentration. All the solutions were filtered using a 0.2 μm syringe filter before HPLC analysis and appropriately degassed using an ultrasonic bath (Hwashin, South Korea) for 15 min. The calculations, such as data acquisition, peak integration, and calibration were performed using Dionex Chromeleon software (version 6.80 RS 10).

2.4. Cell line and cell culture

RAW 264.7 macrophage cells (ATCC® TIB-71™) were purchased from American Type Culture Collection (ATCC, Manassas, Virginia, USA). The cells were cultured in high glucose DMEM supplemented with 10% heat-inactivated bovine serum (FBS) and 1 × Penicillin-Streptomycin solution (Gibco) at 37 °C with 5% CO_2.

2.5. Sulforhodamine B (SRB) assay

The SRB assay was employed by following the previously described protocol with minor modifications to assess the cell viability [30]. RAW 264.7 cells (4×10^4 cells/well) were seeded in a 96-well plate and incubated for 24 h for the cells to adhere. In the presence or absence of rising concentrations (2.5–200 μg/mL) of EPP or 0.5% DMSO (control) for 24 h. After the incubation period, cells were fixed by adding 10% TCA, and the plate was incubated at 4 °C for 60 min. After that, the plate was washed, dried and stained with 0.4% 100 μL of SRB. After another incubation for 30 min, the plate was washed and allowed to dry at room temperature. The protein-bound dye was dissolved in 200 μL of 10 mM Tris base solution (pH 10.0), and the optical density was measured at 515 nm using a microplate reader (BioTeK SynergyMx). The experiments were conducted in triplicate.

2.6. Inhibition of LPS-induced NO generation in RAW 264.7 macrophage cells

The nitrite concentration in the culture medium was measured as an indicator of NO production [15]. In short, RAW 264.7 cells were seeded at a density of 4×10^4 cells/well in phenol red media in a 96-well plate and cultured in a humidified incubator with 5% CO_2 at 37 °C for 24 h. Afterwards, the cells were treated with increasing EPP concentrations (10–40 μg/mL) for 4 h followed by 1 μg/mL LPS treatment for 20 h. When incubation was completed, NO production was spectrophotometrically assessed by measuring nitrite concentrations using the Griess reaction. Absorbance was measured in a microplate reader (BioTeK SynergyMx) at 540 nm against a calibration curve with sodium nitrite standards. The SRB assay assessed cellular viability under a similar experimental set-up.

2.7. Measurement of ROS/RNS level

To measure the formation of intracellular ROS and reactive nitrogen species (RNS) a fluorogenic dye 2′,7′ dichlorodihydro-fluorescein diacetate ($H_2DCF-DA$) was used that detects hydrogen peroxide, peroxyl radicals, and peroxynitrite anions [31]. Briefly, RAW 264.7 cells were seeded into a 96-well plate (Black) with a density of 4×10^4 cells/well and incubated for 24 h to let the cells attach to the bottom. Afterwards, the cells were treated with or without EPP for 4 h before adding 1 μg/mL LPS for additional 20 h. The cells were then washed twice with FBS free media and stained with 10 μM of $H_2DCF-DA$ in FBS free media for 30 min at 37 °C, away from light. After the incubation period, the media with the dye was discarded and cells were washed twice with fresh serum free media. The relative fluorescent intensity of the cell suspensions was measured using a fluorescence plate reader (BioTeK SynergyMx) at an excitation wavelength of 485 nm and an emission wavelength of 520 nm. The SRB assay assessed cellular viability under a similar experimental set-up.

2.8. Western blot

Western blot analysis was carried out employing the previously described method with slight modifications [32]. RAW 264.7 cells were cultured in a 96-well plate with a 1×10^6 cells/well density and allowed to grow until 70–80% confluence. Afterwards, the cells were treated with or without EPP for 4 h before adding 1 μg/mL LPS for additional 20 h. After the incubation period, cells were washed with ice-cold phosphate-buffered saline (PBS), and incubated for 10 min with 100 μL RIPA buffer [150 mM NaCl, 20 mM Tris (pH 7.5), 1 mM ethylenediaminetetra-acetic acid, 1% IGEPAL CA-630, 0.5% sodium deoxycholate, and 0.1% SDS] that contained a protease and phosphatase inhibitor cocktail [0.1 mg/mL phenylmethylsulfonyl fluoride (PMSF)], 30 μL/mL aprotinin, 5 μg/mL leupeptin, and 1 mM sodium orthovanadate. Lysates were collected by a cell scraper and brought into a sterile microcentrifuge tube, and then centrifuged at 15,000×g for 15 min at 4 °C. After collecting the supernatant, protein concentrations were obtained via the Bradford assay. An equal amount of total proteins was separated with SDS-polyacrylamide gel electrophoresis (PAGE) and afterwards electro-blotted onto poly-vinylidene difluoride (PVDF) membranes (BioRad, USA). The blots were incubated with blocking buffer for 1 h at 25 °C, followed by incubation with the appropriate primary antibodies overnight at 4 °C. Then, blots were washed with Tris-buffered saline with 0.1% Tween® 20 (TBST) and incubated with the corresponding secondary antibodies for 1 h. The expression levels of respective proteins were normalized to the level of β-actin. The experiments were conducted in triplicate.

2.9. Animal handling and maintenance

Young Swiss-albino mice comprising of both sexes (22–28 g) were collected from the Animal House, Khulna University, and kept in standard laboratory conditions (at Pharmacology Laboratory, Pharmacy Discipline, Khulna University) for 1 week (temperature 25–28 °C, humidity 55 ± 5% and 12 h light/dark cycle) to ensure better adaptation. All animal experiments were conducted following the guidelines of the Animal Ethics Committee, Khulna University Research Cell, Khulna 9208, Bangladesh, and the reference number is KUAEC-2021/06/10.

2.10. In-vivo acute toxicity study

Acute toxicity test was conducted according to the Organization for Economic Co-operation and Development (OECD) guidelines-425 with brief modifications [33]. 20 experimental animals were randomly chosen and divided into four groups: Control (2% tween-80 in water), EPP 1 g/kg, EPP 2 g/kg, and EPP 3 g/kg. After the oral administration of the respective doses, animals were observed individually for the initial 30 min and then at every 24 h for 14 days to identify any clinical signs of toxicity or mortality.

2.11. Sub-acute toxicity study

The sub-acute toxicity test of EPP was performed according to the procedure previously described with brief modifications [33]. 10 experimental animals were randomly chosen and divided into two groups: Control (2% tween-80 in water) and EPP 500 mg/kg. The mice daily received respective doses orally for 14 days. After 14 days of completion, the animals were anesthetized, and their blood samples were collected from the cervical vein [34]. For biochemical analyses, blood without additive was centrifuged at 1500 rpm for 20 min, serum was separated and SGPT, SGOT, ALP, bilirubin, creatinine, urea, total cholesterol, and triglyceride were estimated using a blood analyzer [35].

2.12. Optimum dose selection

During the sub-acute toxicity study, the test group was treated with 500 mg/kg EPP for 14 days. After the treatment period, when mice blood samples were analyzed, the above mentioned serum biochemical parameters, including SGPT, SGOT, creatinine, bilirubin, etc. did not show any adverse fluctuation. Thus it suggests, 500 mg/kg dose of EPP is safe to administer in mice for longer term. Hence, 500 mg/kg was selected as the highest dose for all the *in-vivo* experiments.

2.13. Formaldehyde-induced edema test

The anti-inflammatory activity was assessed following the previously mentioned protocol with brief modifications [36]. 30 mice of both sexes were distributed into four groups: Control (2% Tween-80 in water), Ibuprofen 10 mg/kg, EPP 250 mg/kg, and EPP 500 mg/kg oral dose. All the animals were treated with 0.2% of 0.01 mL formaldehyde using sub-plantar injection in the right paw after 60 min of the oral administration of the previously mentioned doses. After 1, 2, 3, 4, and 5 h of administration of ibuprofen and EPP, the paw volume of mice was quantified using a millimeter slide calipers. The percent inhibition of inflammation by EPP was measured through the following equation $= [(V_c\text{-}V_t)/V_c \times 100]$. Here, V_c – average inflammation induced in the control group, and V_t – average inflammation induced in ibuprofen and EPP treated groups.

2.14. Anti-allergic activity test

2.14.1. TDI sensitization and provocation
The entire test was conducted as per procedure previously described with slight modifications [37,38]. Experimental animals were arbitrarily chosen and divided into five groups: Control, TDI-control, Cetirizine 20 mg/kg, EPP 300 mg/kg, and EPP 500 mg/kg 10 μL of 5% TDI solution dissolved into ethyl acetate was applied bilaterally on the nasal vestibule of each mouse group except for the control group who only received the vehicle ethyl acetate (10 μL). All the groups received respective doses of treatments orally, only the control group received 2% tween-80 in water. The TDI provocation and the whole experimental protocol [33] are described in S. Fig. S1.

2.14.2. Allergy-like symptoms assessment
On the 21st day, after EPP supplementation, nasal allergy-like symptoms were recorded just after TDI provocation and assessed carefully for 10 min by accommodating the animals into different cages [38]. The extent of watery rhinorrhea was evaluated on a grading scale ranging from 0 to 3 (S. Table S1).

2.14.3. Blood and bronchoalveolar lavage (BAL) fluid collection and analysis
After 24 h of TDI provocation, animals of each group were anesthetized by injecting sodium pentobarbital. Blood and BAL fluid collection were conducted as per the previously described method [39]. Blood was collected from the cervical vein, immediately transferred into heparinized tube, and diluted at 1:10 ratio with 1% acetic acid to lyse the red blood cells. The total WBC count was conducted using an automated cell counter (DS-500i, 5 parts automated hematology analyzer; Edan Instruments Inc., Shenzhen, China). For differential WBC counts, Leishman-stained slides were analyzed (300 cells/slide) via a compound microscope (oil immersion power) at $400 \times$ magnification. BAL fluid was collected using 0.9% sterile saline which was introduced into lung via cannulated tracheal tube. Collected fluids were centrifuged at 3500 rpm for 15 min to separate the supernatant. The settled cells were washed with 250 μL of 0.9% saline containing bovine serum albumin (BSA) and finally taken for total and differential cell count using above mentioned methods.

2.15. In-silico molecular docking simulation

Syringic acid was identified from EPP via HPLC-DAD dereplication analysis and its structure was optimized using Avogadro (version 1.2.0), until the atomic/angle stable conformation was obtained [40]. The crystal structures of the H1R and iNOS were extracted from the Protein Data Bank (RSCB PDB ID: 3RZE [41] and 4NOS [42], respectively). Based on the 7 major aromatic interaction points i.e., Tyr 108, Phe 184, Phe 190, Phe 199, Phe 424, Trp 428, and Tyr 431 of H1R which are vital for the formation of lipophilic pocket of the antagonist binding cavity [43], the entire sequence was counted for further analysis and the oxygen atoms were deleted via PyMol (version 1.3) software package [44]. After that, Swiss-PDB viewer (version 4.1.0) was employed to optimize the protein structures that ensured minimum energy. The typical non-bonding interactions and binding affinities of the ligand with H1R and iNOS were calculated using Autodock Vina software package for the docking analysis [45]. Finally, syringic acid was analyzed and visualized with H1R and iNOS through PyMOL Molecular Graphics System [44] and Discovery Studio (version 4.1) [46].

2.16. Statistical analysis

All data were shown as mean ± Standard error mean (SEM) except for the *in-vitro* assays (mean ± SD). All statistical analyses were conducted using one-way ANOVA followed by Dunnet's multiple comparisons using SPSS Data Editor for Windows (version 16.0) (SPSS Inc., USA). All the graphs were generated and presented using GraphPad Prism (version 8.0.2). The *p* values < 0.05 were counted as statistically significant.

3. Results

3.1. Quantification of polyphenolic metabolites using HPLC-DAD

HPLC-DAD analysis of EPP has shown the notable existence of syringic acid (89.19 mg/100 g dry extract). Moreover, *p*-coumaric acid, *trans*-ferulic acid, rosmarinic acid, myricetin, and kaempferol were also found in different amounts (Figs. 1A, B and 2).

3.2. LPS-induced NO generation assay in raw 264.7 cells

NO, a free radical that is synthesized from L-arginine via the activity of the nitric oxide synthase (NOS) enzymes family: endothelial NOS (eNOS), neuronal NOS (nNOS), and iNOS. The generation of NO is greatly involved in the pathogenic pathway of inflammation as it presents the cells with considerable amount of oxidative stress [16]. EPP exhibited dose-dependent inhibition of LPS-induced NO production in RAW 264.7 cells with the highest dose of 40 µg/mL, showing 72.41% inhibition ($p < 0.001$) when compared to the LPS-control group. The median inhibitory concentration (IC_{50}) of EPP was calculated to be 28.20 ± 0.27 µg/mL (Fig. 3A).

3.3. H_2DCF-DA ROS/RNS detection

LPS is responsible for generating a wide variety of free radicals, i.e., hydroxyl ions (HO^-), superoxide (O_2^-), etc. that initiate oxidative stress [47]. In this assay, the detection and quantification of ROS/RNS generated corresponded to the fluorescence intensity exerted by H_2DCF-DA. Here, EPP demonstrated inhibition of LPS-induced ROS/RNS generation in a concentration-dependent manner as well ($IC_{50} = 29.47 \pm 2.19$ µg/mL). At its highest dose of 40 µg/mL, EPP significantly ($p < 0.001$) diminished LPS-stimulated ROS/RNS production in RAW 264.7 cells, as evidenced by the decrease in fluorescence intensity (Fig. 3B). Compared to the
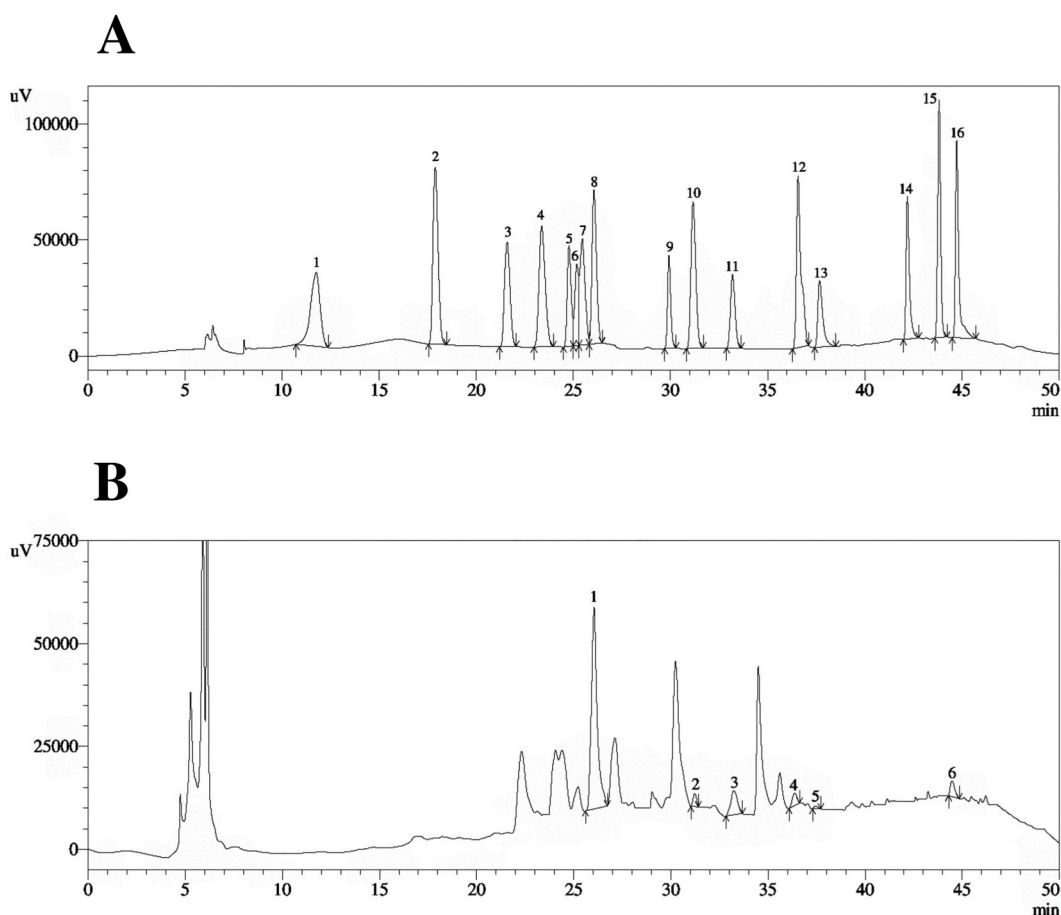

Fig. 1. (A) HPLC chromatogram of a standard mixture of polyphenolic compounds peaks: 1, gallic acid; 2, 3,4 dihydroxy benzoic acid; 3, catechin hydrate; 4, catechol; 5, (−) epicatechin; 6, caffeic acid; 7, vanillic acid; 8, syringic acid; 9, rutin hydrate; 10, *p*-coumaric acid; 11, *trans*-ferulic acid; 12, rosmarinic acid; 13, myricetin; 14, quercetin; 15, *trans*-cinnamic acid; 16, kaempferol, and (B) HPLC chromatogram of EPP peaks: 1, Syringic acid; 2, *p*-coumaric acid; 3, *trans*-ferulic acid; 4, rosmarinic acid; 5, myricetin; and 6, kaempferol. EPP = Ethanolic leaf extract of *P. pubinerve*.
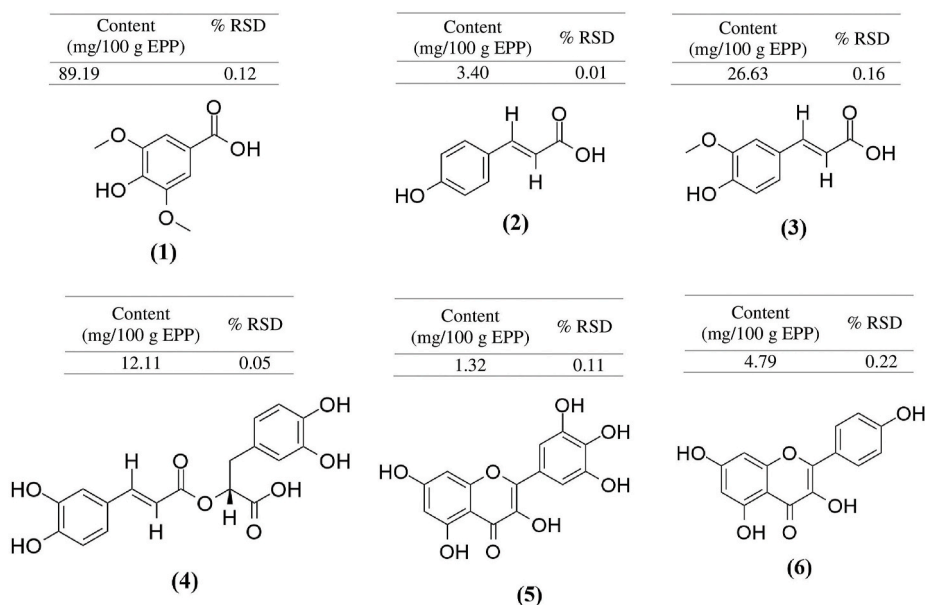
Content (mg/100 g EPP)	% RSD
89.19	0.12

(1)

Content (mg/100 g EPP)	% RSD
3.40	0.01

(2)

Content (mg/100 g EPP)	% RSD
26.63	0.16

(3)

Content (mg/100 g EPP)	% RSD
12.11	0.05

(4)

Content (mg/100 g EPP)	% RSD
1.32	0.11

(5)

Content (mg/100 g EPP)	% RSD
4.79	0.22

(6)

Fig. 2. Chemical structures of identified polyphenolic compounds by HPLC-DAD dereplication and quantification of their corresponding contents. Here, (1) syringic acid, (2) *p*-coumaric acid, (3) *trans*-ferulic acid, (4) rosmarinic acid, (5) myricetin, and (6) kaempferol.
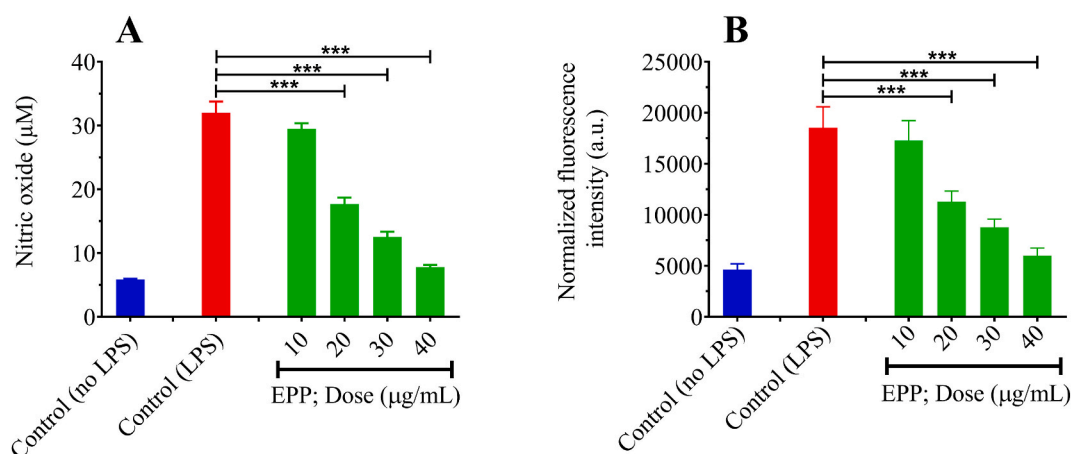
Fig. 3. (A) Inhibition of NO production at different concentrations of EPP (10–40 μg/mL) was assayed in *in-vitro* LPS (1 μg/mL) induced RAW 264.7 cells, and (B) suppression of intracellular ROS and RNS production by EPP at different concentrations (10–40 μg/mL) was studied using H_2DCFDA fluorogenic dye in LPS (1 μg/mL) induced RAW 264.7 cells. EPP = Ethanolic leaf extract of *P. pubinerve*. The results are expressed as the mean ± SD (n = 3). One-way ANOVA indicated, *$p < 0.05$, **$p < 0.001$ and ***$p < 0.0001$ as statistically significant compared to respective control groups (Dunnett's test).

LPS-control group, we observed a 3.77-fold decrease in fluorescence intensity in the 40 μg/mL EPP treated group (67.43% inhibition). This depletion of ROS/RNS generation supports the efficacy of EPP as a potential agent against allergic inflammation [48].

3.4. Western blot

The protein expressions of iNOS, COX-2, NF-κB, IL-6, and TNF-α in RAW 264.7 cells were evaluated after treatment with different EPP concentrations (10–40 μg/mL), followed by stimulation with LPS to further investigate the mechanistic role of the inflammatory proteins that are implicated in the pathogenesis of allergic inflammation. Western blotting demonstrated that EPP at its highest concentration significantly ($p < 0.001$) downregulated the expressions of iNOS (2.7-fold decrease) (Fig. 4B). The downregulation of iNOS is consistent with our previous outcome found in the NO inhibition assay, where EPP suppressed NO generation in RAW 264.7 cells. The inflammatory protein COX-2 is overexpressed during allergic inflammation as it modulates the activity of prostaglandin and thromboxane in the nasal airway [49]. It was observed that COX-2 expression was notably suppressed by EPP (3.2-fold decrease at 40
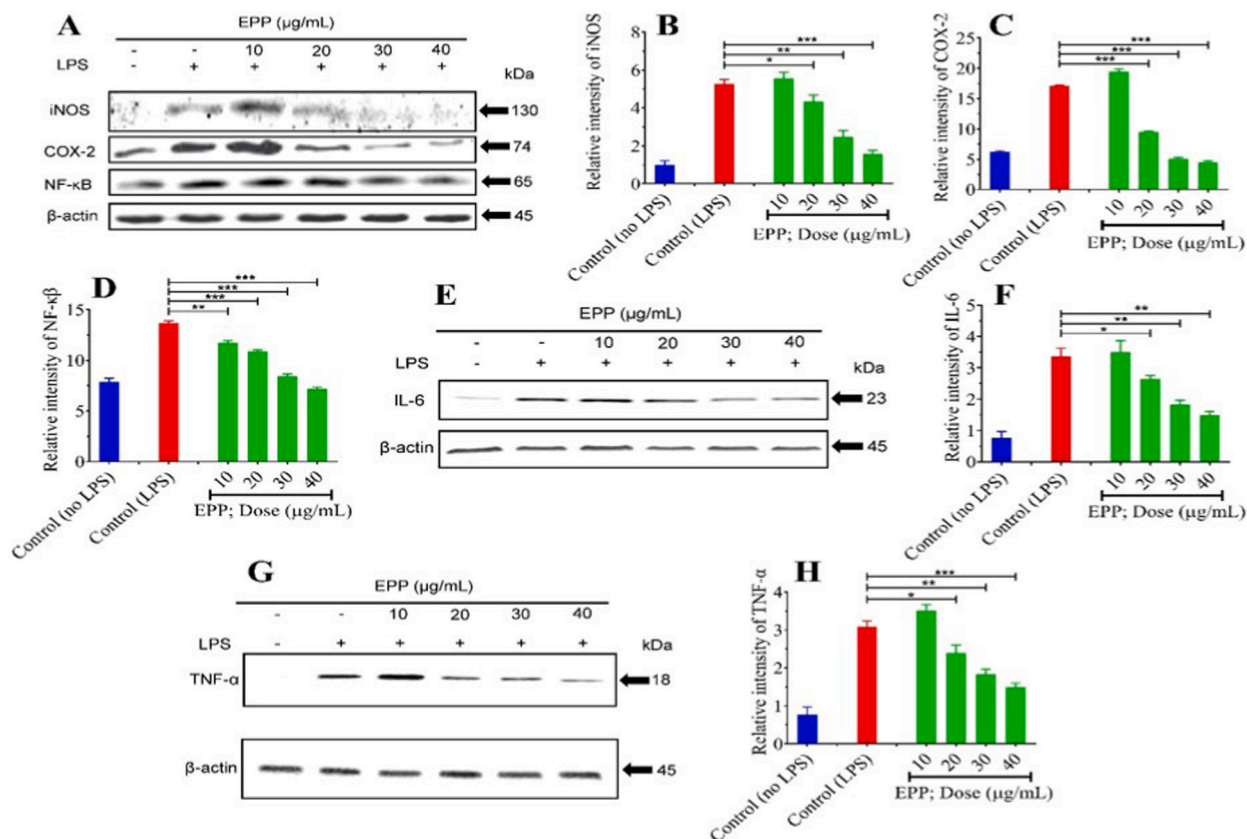
Fig. 4. (A) EPP downregulated the expressions of iNOS, COX-2, NF-κB, (E) IL-6, and (G) TNF- α in RAW 264.7 cells. Western blot analysis was conducted for the proteins of interest in RAW 264.7 cells treated with the different concentrations of EPP (10–40 µg/mL) in presence of LPS. RAW 264.7 cells without EPP treatment (with or without LPS) in the growth medium were considered as controls in each protein expression analysis. 1 µg/mL concentration of LPS upregulated iNOS, COX-2, NF-κB, IL-6, and TNF- α expressions. Relative changes of iNOS, COX-2, NF-κB, IL-6, and TNF-α expressions are shown as a function of EPP concentration. The relative intensity of (B) iNOS, (C) COX-2, (D) NF-κB, (F) IL-6, and (H) TNF- α decreased as the concentration of EPP gradually increased to 40 µg/mL. The band intensity was adjusted by the corresponding β-actin band intensity. EPP = Ethanolic leaf extract of *P. pubinerve*. All data are presented as the mean ± SD (n = 3). One-way ANOVA indicated, $*p < 0.05$, $**p < 0.001$ and $***p < 0.0001$ as statistically significant compared to respective control groups (Dunnett's test). Full and non-adjusted images of all the blots are provided in the supplementary material (S. Figs. S7–10).

µg/mL) (Fig. 4C). EPP abolished the NF-κB activation in RAW 264.7 cells by downregulating the expression of p65 (1.5-fold decrease at 40 µg/mL) (Fig. 4D), suggesting its promising potential in tackling allergy-associated inflammation [13]. The imbalance in T_H1 and T_H2 system is responsible for inducing allergic inflammation [50]. Therefore, the levels of T_H2 cytokine IL-6 and T_H1 cytokine TNF-α were also assessed. Both of the cytokine levels reduced significantly at the highest EPP dose, 40 µg/mL (Fig. 4E–H), which further ascertained the role of EPP against allergic inflammation.

3.5. In-vivo acute toxicity study

EPP was observed non-toxic in the acute toxicity test as there was no abnormal sign of physiological and behavioral change even with the highest dose group, 3 g/kg (S. Table S2). There was a consistent rise in body weight of mice in all the test groups in addition to no observed lethality (Fig. 5A). Hence, it can be estimated that the LD_{50} of EPP is above 3 g/kg.

3.6. In-vivo sub-acute toxicity study

Serum biochemical parameters are handy in detecting abnormalities in specific organ functions, such as the levels of SGPT, SGOP, and ALP in serum are indicative of liver health [51]. It can be observed that no serum parameter in the 500 mg/kg group varied significantly when compared with the control group (Fig. 5B, C). This suggests that EPP did not cause any organ-specific, i.e., liver, kidney, heart, etc. harm to mice.
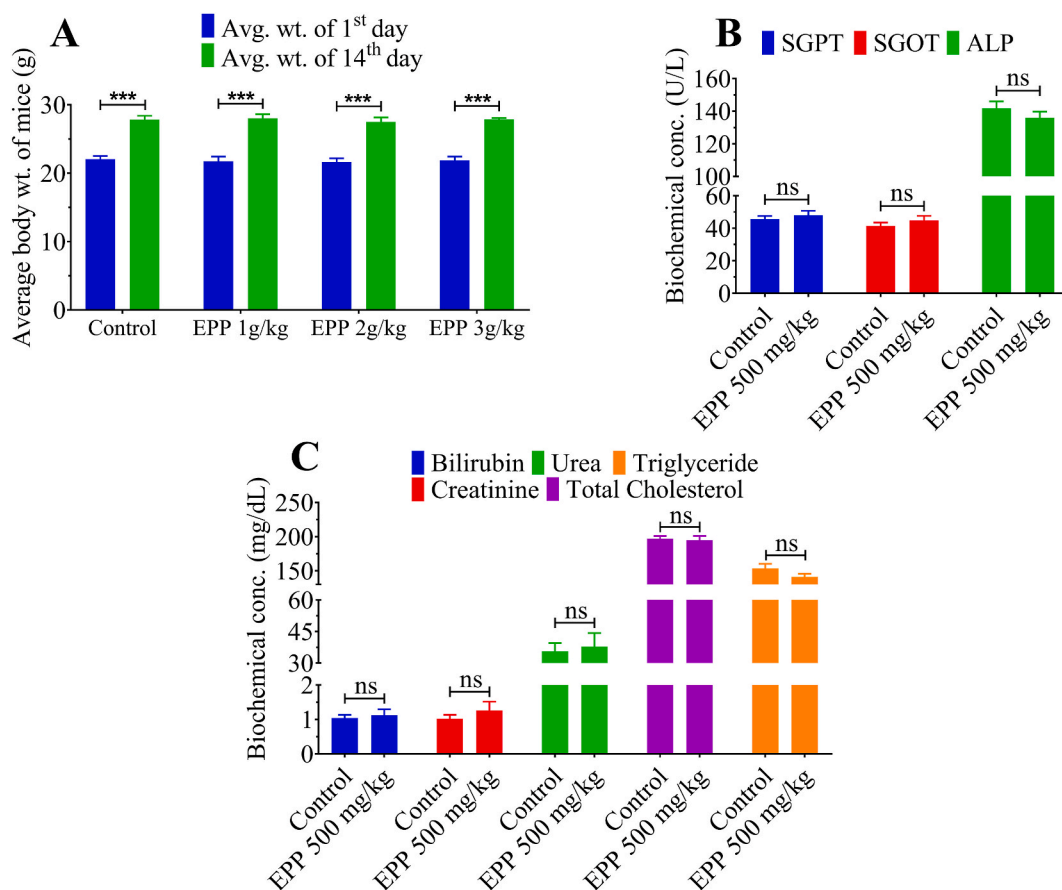
Fig. 5. (A) Effect of EPP on average mice body weight (acute toxicity study), and (B) & (C) Effect of EPP on different biochemical parameters in mice blood (sub-acute toxicity study). The results are expressed as the mean ± SEM, (n = 5). EPP = Ethanolic leaf extract of *P. pubinerve*, and ns = not significant. In Fig. 5(A), ***$p < 0.0001$ vs. avg. wt. of 1st day (Dunnett's test).

3.7. In-vivo anti-inflammatory study

Formaldehyde-mediated paw edema is associated with substance P (SP), a neurotransmitter distributed throughout the central and peripheral nervous system [52]. The release of prostaglandin E$_2$ (PGE2) is another physiological response to formaldehyde induction into body systems that causes swelling, redness, and pain [53]. EPP decreased the paw volume of mice in both 250 mg/kg and 500 mg/kg doses (Fig. 6A–E). After 5 h, the 500 mg/kg test group was able to cause 66.41% inhibition of inflammation which was comparable with standard drug ibuprofen's percent inhibition of inflammation, 76.31%. As COX has a cornerstone role in developing the later phase of inflammation via converting arachidonic acid into prostaglandin, inhibition of COX by EPP is expected to be mechanistically involved behind this effect. Furthermore, systemic and local inflammations are mediated by cytokines like TNF-α, IL-1, IL-6, H1R, iNOS, etc. [54,55]. Therefore, EPP-mediated amelioration of inflammation might involve the downregulation of these proteins.

3.8. In-vivo anti-allergic activity study

Allergic inflammatory symptoms, such as sneezing, scratching, nasal scores, etc. In nasal mucosa are vital in rhinitis [56]. Oral administration of EPP at 500 mg/kg doses significantly lessened the number of sneezes, scratches, and nasal scores (17.2 ± 0.99, 152 ± 3.56, 1.6 ± 0.2, respectively) which is comparable to that of the standard drug, cetirizine 20 mg/kg group (14.5 ± 0.8, 128.2 ± 5.55, 1.3 ± 0.26, respectively) (Fig. 7A, B, C). Reportedly, the number of total and differential inflammatory WBCs rise during allergic inflammation to fight the deleterious roles of the exogenous antigens and build effective immunological response towards the same [57,58]. The number of circulating WBC, lymphocytes, neutrophils, eosinophils, monocytes, and basophils were significantly reduced in the EPP treated 500 mg/kg test group which was comparable to the action of the cetirizine 20 mg/kg group (Fig. 8A–F). A quite similar experimental outcome was observed while BAL fluids of different groups were analyzed in terms of total and differential WBC (Fig. 9A–F). Collectively, treatment of EPP with 500 mg/kg dose caused notable symptomatic and systemic relief from allergic inflammation.
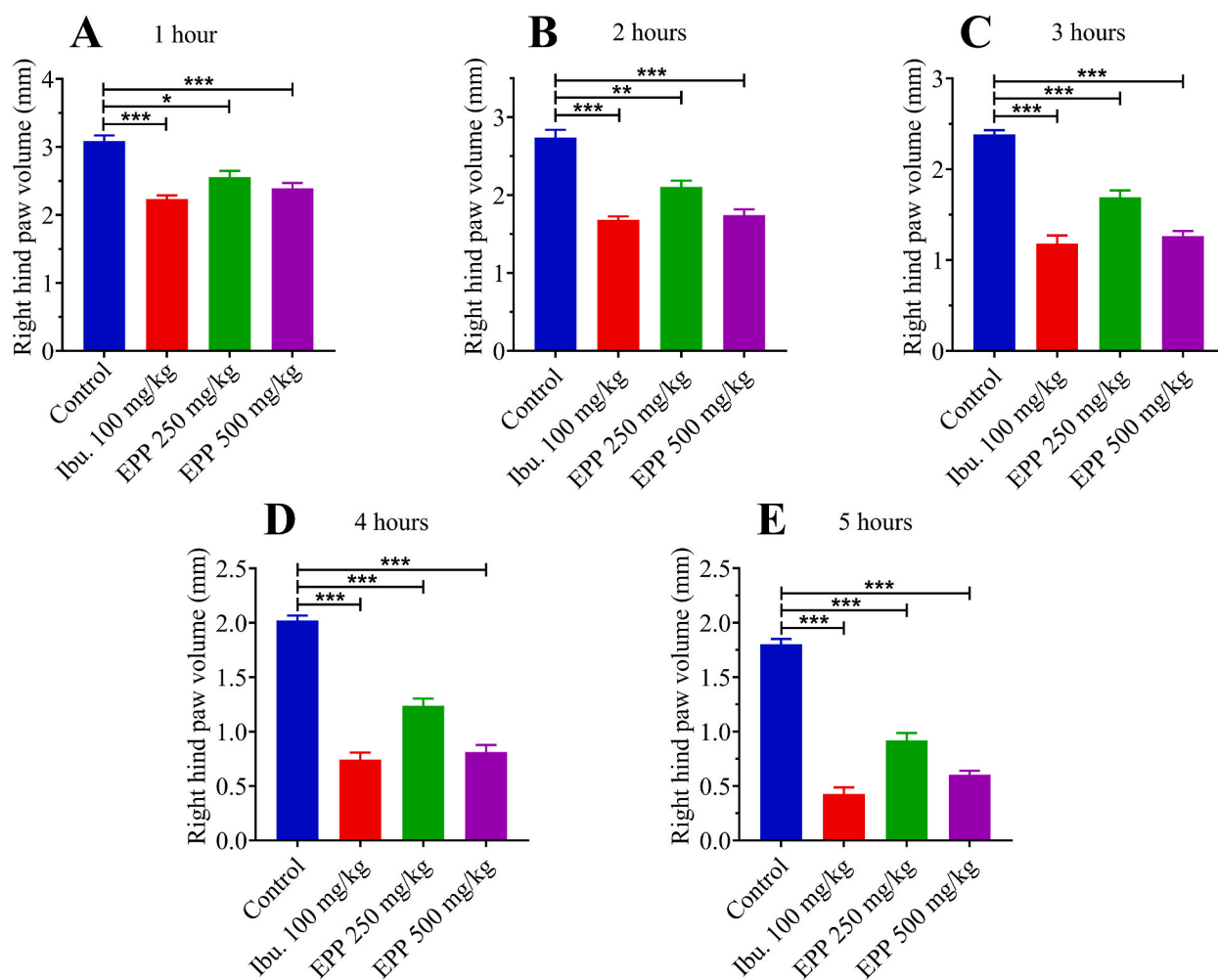
Fig. 6. EPP attenuated formaldehyde induced paw edema volume in mice after 1–5 h, described in the figure as A-E). The results are expressed as the mean ± SEM, (n = 6). EPP = Ethanolic leaf extract of *P. pubinerve*, and Ibu. = Ibuprofen. Here, *$p < 0.05$ vs. Control (Dunnett's test); **$p < 0.001$ vs. Control (Dunnett's test); ***$p < 0.0001$ vs. Control (Dunnett's test).

3.9. In-silico molecular docking simulation

After the molecular docking of syringic acid with H1R and iNOS, the lowest binding affinities against these proteins were −6.6 and −6.7 kcal/moL, respectively (Fig. 10 A-D, 11 A-D). During intermolecular nonbonding interactions with H1R, syringic acid bound with TYR108 and TYR431 amino acid residues (Fig. 10B, C), which are among the 7 major aromatic interaction points important for lipophilic pocket formation during antagonist binding [43]. During its binding with iNOS, syringic acid formed H-bond with TRP463 (Fig. 11B, C), which is one of the four active site residues (Trp372, Glu377, Trp463, Phe476) of iNOS [59]. Syringic acid formed 8 and 7 H-bonds with H1R and iNOS, respectively which suggests the availability of abundant number of –OH groups attached to the aromatic ring in its structure for potential interaction with the receptor proteins (Figs. 10D, 11D). One of the major prospects for syringic acid as a potential binding agent to work as an antagonist of H1R and iNOS is its high number of H-bond donors and acceptors, which is 2 and 5, respectively. As a comparatively smaller molecule with one aromatic ring, syringic acid demonstrated relatively satisfactory binding affinities towards H1R and iNOS, when compared to the standard drug cetirizine used in the *in-vivo* study (−8.6 kcal/moL towards H1R and −9.4 kcal/moL towards iNOS) (data not shown). Compared to syringic acid, *trans*-ferulic acid and rosmarinic acid showed lower binding affinities against H1R (−7.3 and −8.6 kcal/moL, respectively) and iNOS (−7.7 and −8.6 kcal/moL, respectively) (S. Figs. S2–5). The probable reason might be, these molecules contain a greater number of H-bond interaction sites compared to syringic acid, i.e., rosmarinic acid contains 5 H-bond donors and 8 H-bond acceptors.

4. Discussion

Allergic inflammation can lead to severe outcomes, including chronic and acute sinusitis, bronchial asthma, upper and lower nasal
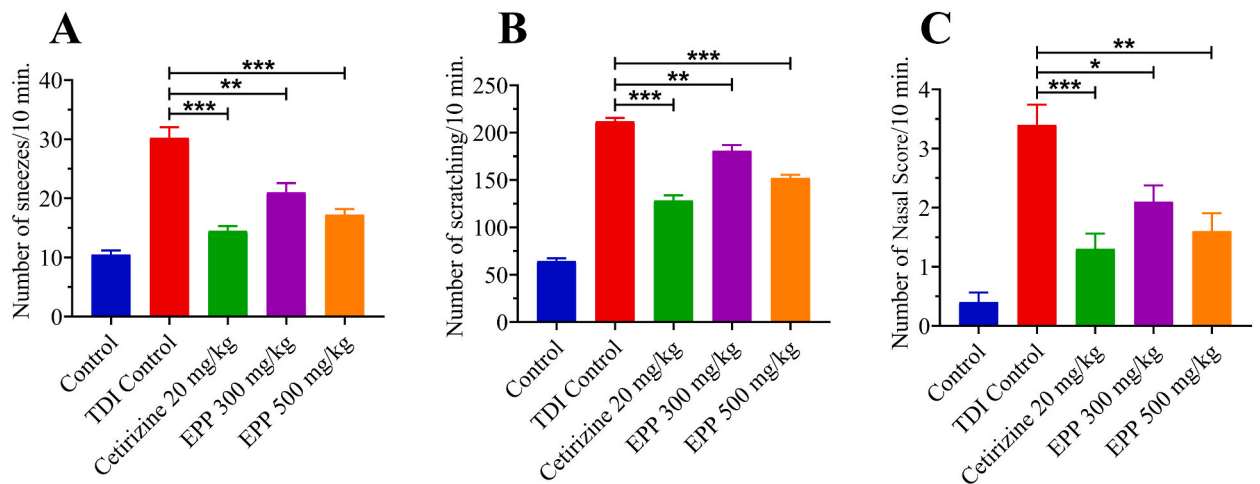
Fig. 7. EPP alleviated allergic symptoms, such as (A) sneezing, (B) scratching, and (C) nasal scores in mice. The results are expressed as the mean ± SEM, (n = 10). EPP = Ethanolic leaf extract of *P. pubinerve*. Here, $*p < 0.05$ vs. TDI control (Dunnett's test); $**p < 0.001$ vs. TDI control (Dunnett's test); $***p < 0.0001$ vs. TDI control (Dunnett's test).
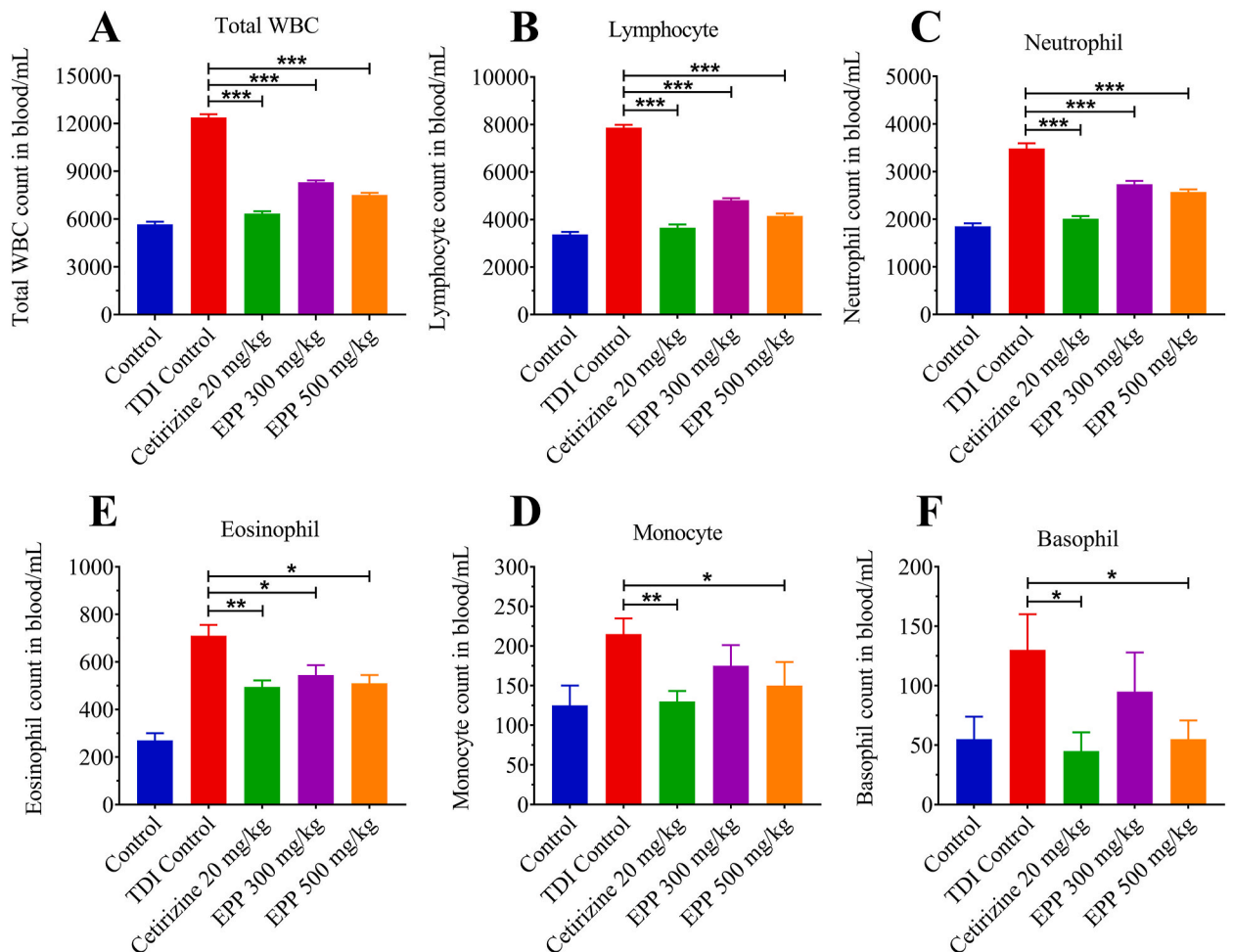

Fig. 8. EPP reduced the number of total and differential WBC in mice blood. The results are expressed as the mean ± SEM, (n = 10). EPP = Ethanolic leaf extract of *P. pubinerve*. Here, $*p < 0.05$ vs. TDI control (Dunnett's test); $**p < 0.001$ vs. TDI control (Dunnett's test); $***p < 0.0001$ vs. TDI control (Dunnett's test).
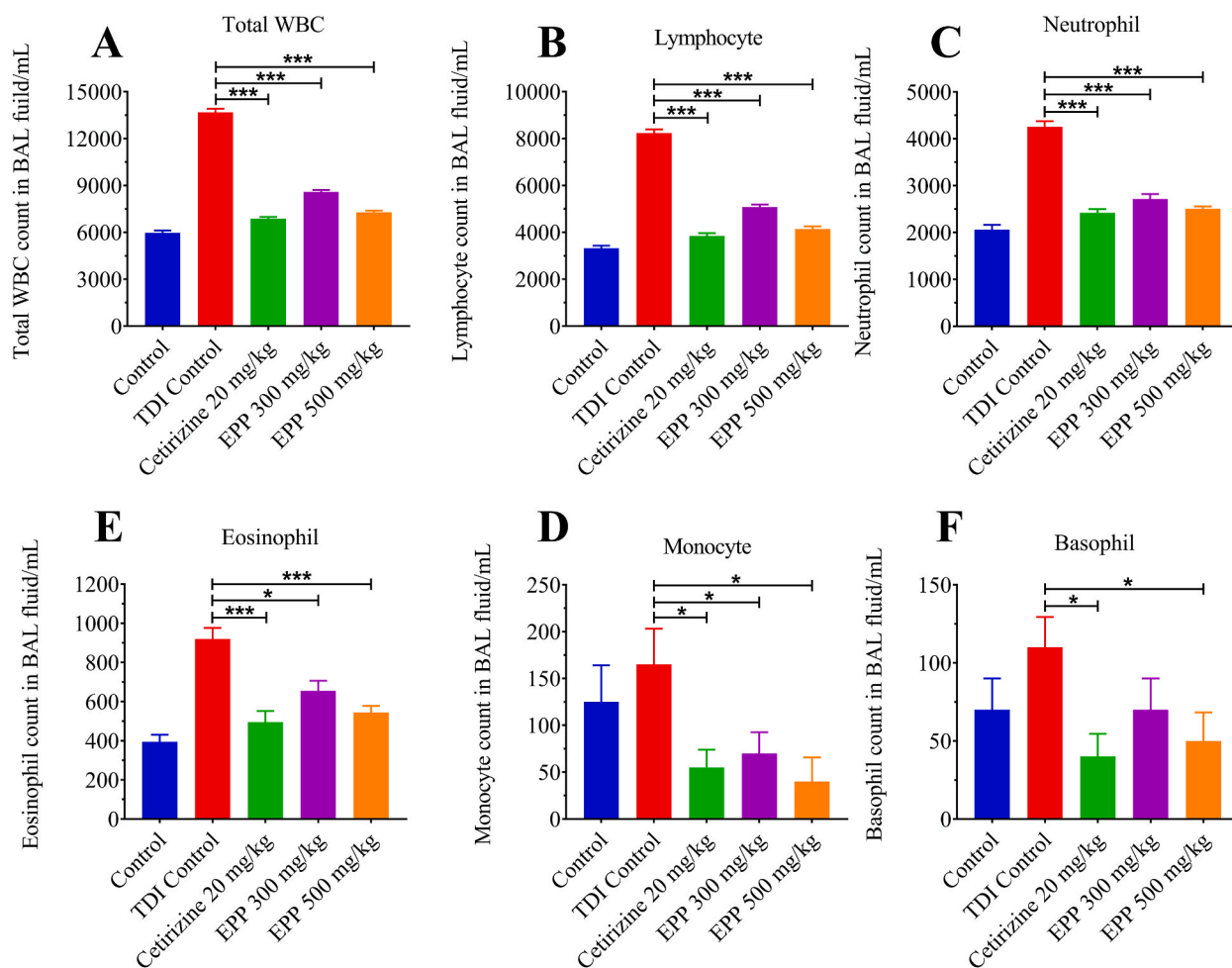
Fig. 9. EPP reduced the number of total and differential WBC in mice BAL fluid. The results are expressed as the mean ± SEM, (n = 10). EPP = Ethanolic leaf extract of *P. pubinerve,* and BAL fluid = Bronchoalveolar lavage fluid. Here, *$p < 0.05$ vs. TDI control (Dunnett's test); **$p < 0.001$ vs. TDI control (Dunnett's test); ***$p < 0.0001$ vs. TDI control (Dunnett's test).

airway complications, coronary instability, etc. If kept untreated [60–63]. Not to mention, treatment options for these diseases are not only expensive and time-consuming, but they require multiple medications. In our study, the HPLC-DAD dereplication analysis showed the presence of a substantial amount of syringic acid (Fig. 1A, B, 2). It is previously reported, syringic is a potent antioxidant and thus can scavenge the deleterious actions of ROS and RNS [64]. Moreover, it was found promising in downregulating the inflammatory cytokines, including IL-4, IL-5, IL-13, TNF-α, and the inflammatory cells, such as eosinophils, neutrophils, macrophages, and lymphocytes in the BAL fluid of asthma induced mice [65]. IgE, responsible for mediating allergic inflammation was found to be suppressed in the syringic acid-treated mice group, according to the same study. Syringic acid was also previously observed to downregulate inflammatory signaling proteins, including iNOS, COX-2, and NF-κB [66] which is in line with our findings obtained from the Western blot analysis (Fig. 4). In its structure, syringic acid has a benzene ring linked with one –OH, two –OCH$_3$, and one –COOH group. The ROS/RNS scavenging property of syringic acid is dependent on the number of –OH groups linked with the aromatic ring and because of the presence of –OH in the expected area, syringic acid is more active in scavenging free radicals than other phenolic acids [66]. In addition, the presence of two –OCH$_3$ moieties at positions 3 and 5 of the aromatic ring enhances the free radical scavenging property of syringic acid [67]. Furthermore, it reportedly inhibited the deleterious roles of HO•, HOO•, and NO$_2$ free radicals [64]. These collective roles of syringic acid might be responsible for EPP's action against allergic inflammation.

LPS-induced NO generation was remarkably suppressed by EPP in RAW 264.7 cells (Fig. 3A). Characteristically, NO is known for its obnoxious free radical generating property and is involved in allergic inflammation and bronchial hyper-responsiveness [68,69]. Enhanced NO level is correlated with increased serum IgE level as well [70]. The ROS/RNS were also downregulated by EPP as evidenced by the measurement of ROS/RNS in LPS-induced RAW 264.7 cells (Fig. 3B). Reportedly, ROS/RNS are responsible for inducing pro-inflammatory reactions in nasal airways via antioxidant depletion and contribute to exacerbating allergic inflammation [48]. The ROS/RNS scavenging property of EPP might be associated with antioxidative signaling pathways, like kelch-like ECH-associated protein 1(Keap1)-nuclear factor erythroid 2-related factor 2 (Nrf2)-antioxidant response element (ARE), Nrf2-h*eme*
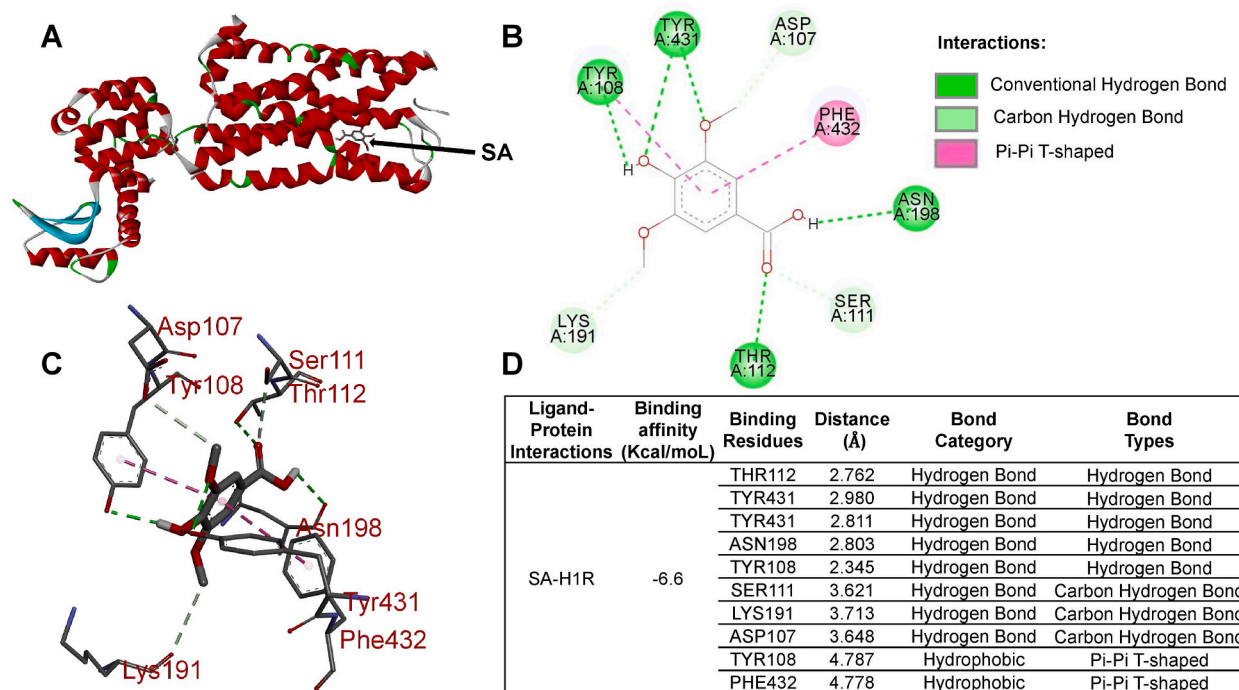
Fig. 10. Molecular docking and ligand-protein binding interactions between SA and H1R. Here, (A) represents the molecular docking of SA with H1R. (B) represents the 2D diagram of nonbonding interactions between SA and H1R. (C) represents the amino acid residues of H1R involved in ligand-protein nonbonding interactions in 3D diagram, and (D) illustrates the binding affinity, bound amino acid residues, and bond distances, category, and types formed between SA and H1R. SA = Syringic acid.

*oxygenase-1 (*HO-1), etc. which dictate the balancing mechanism between free radicals and antioxidants within the body system [71]. The antioxidative property of EPP might also be correlated with stabilizing the functions of mast cells and downregulating the gene expression of IL-4 [72,73]. During Western blot analysis, EPP at its highest concentration downregulated iNOS, COX-2, and NF-κB signaling in RAW 264.7 cells (Fig. 4A–D). Reportedly, LPS, TNF-α, and ROS are responsible for inducing iNOS expression via activating the NF-κB, and inhibition of iNOS essentially results from the inhibition of NF-κB activation [74]. Therefore, the observed down-regulation of iNOS during western blotting is consistent with the suppression of NF-κB expression (Fig. 4 D). In living systems, iNOS, being responsible for NO production facilitates different inflammatory and autoimmune lesions accompanied by activated macro-phages and neutrophils [75]. Therefore, suppression of iNOS expression by EPP suggests its potential in abrogating the inflammatory cells and pathways essential in allergic inflammation. NF-κB is activated via two major signaling mechanisms: the canonical and non-canonical pathways, among which the non-canonical one is stimulated by external pathogens or chemicals and is responsible for inducing inflammation. The dysregulated non-canonical activation of NF-κB leads to abnormal self-reactive B cells survival which causes the generation of autoimmune antibodies associated with various inflammatory conditions [76]. In addition, the abnormal non-canonical NF-κB signaling in endothelial cells can cause excessive generation of chemokines and inflammatory cells, leading to chronic tissue damage and inflammation. This mechanistic analysis suggests that EPP has ameliorated inflammation by down-regulating the NF-κB signaling. Furthermore, the suppression of NF-κB in attenuating allergic conditions is already reported [13]. Hence, the role of EPP against allergic inflammation might be induced by downregulated signaling of NF-κB. In addition, COX-2 inhibitors are already well-established anti-inflammatory drugs that act by blocking the arachidonic acid metabolism and prosta-glandin synthesis. However, the most abundant prostanoid in the body, PGE2 is responsible for enhancing IgE generation by B cells [77]. Hence, inhibition of COX-2 expression by EPP is proposed to be associated with not only its anti-inflammatory role but anti-allergic role as well. In addition, the levels of major cytokines, including IL-6 and TNF-α were also significantly decreased by EPP (Fig. 4E–H). The generation of TNF-α takes place at the initial stage of allergen sensitization, and it continues to facilitate the in-flammatory cascade during the effector phase of allergic reactions [78]. Increased IL-6 expression level was previously found in bronchial epithelial cells, BAL fluid, and peripheral blood of patients with allergic conditions [79]. Hence, downregulation of both of these cytokines by EPP might suggest its role in attenuating the early and also the later phase of allergic inflammation.

The safety of EPP in the living systems was confirmed by the absence of mortality, and abnormal changes in body weight, phys-iological and behavioral features up to 3 g/kg dose in mice (S. Table S2, Fig. 5A). In addition, the non-harming nature of EPP towards different organs, including liver, kidney, and heart at 500 mg/kg dose was evidenced in the sub-acute toxicity study (Fig. 5B, C). Formaldehyde-stimulated paw edema in mice was significantly attenuated by EPP (Fig. 6A–E). During the onset of inflammation, cells exhibit increased permeability and vasodilatation around the inflamed area, expediting the nuclear translocation of various inflam-matory mediators i.e., NF-κB, iNOS, TNF-α, IL-1, IL-4, IL-6, etc. and increase the number of different WBCs [80]. Therefore, EPP might
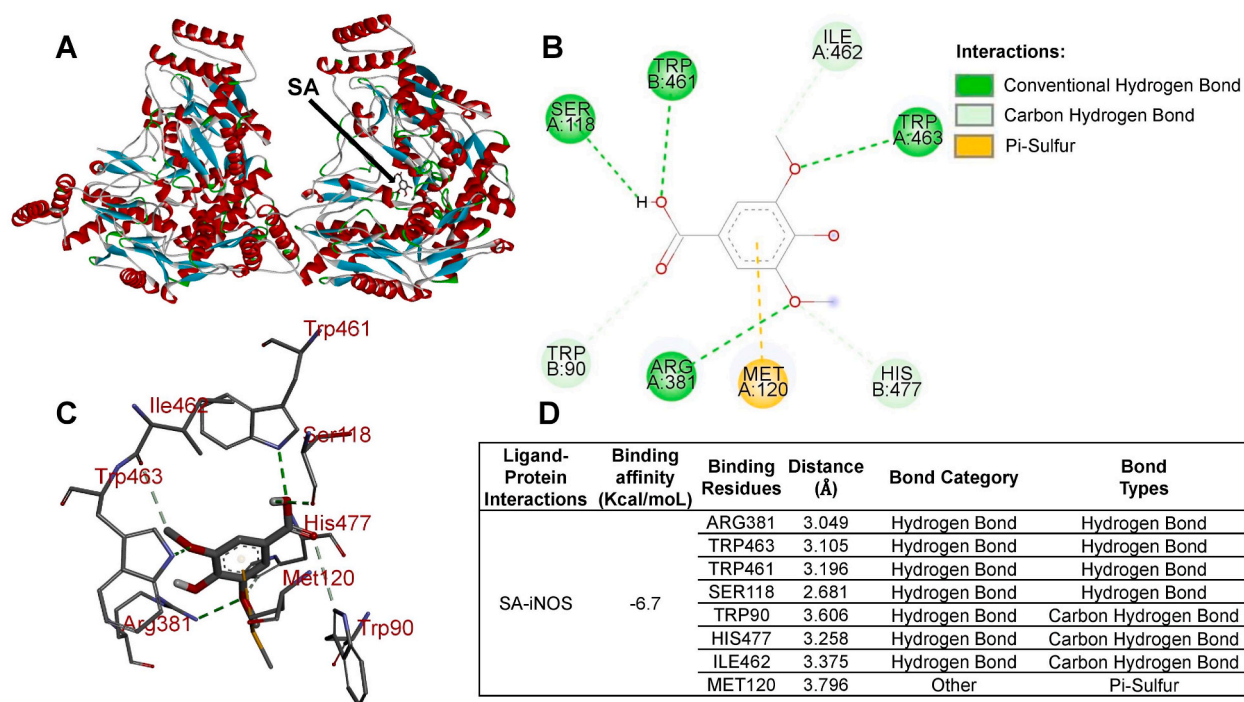
Fig. 11. Molecular docking and ligand-protein binding interactions between SA and iNOS. Here, (A) represents the molecular docking of SA with iNOS. (B) represents the 2D diagram of non-bonding interactions between SA and iNOS. (C) represents the typical ligand-protein nonbonding interactions in 3D diagram, and (D) illustrates the binding affinity, bound amino acid residues, and bond distances, category, and types formed between SA and iNOS. SA = Syringic acid.

exert its action against inflammation via abolishing these cytokine expressions and reducing different inflammatory WBCs, including eosinophil, basophil, neutrophil, etc. [81,82]. In addition, histamine is also one of the major players to initiate and cause inflammation where it upregulates various ILs [83]. As described previously, H1R is one of the receptors of histamine that is involved in the occurrences of inflammation [6]. Thus, suppression of inflammation by EPP might also be accompanied by reduced expressions of histamine and its H1R.

TDI-induced allergic symptoms in mice dropped significantly in the 500 mg/kg EPP treated group (Fig. 7A, B, C). Patients with symptoms of allergic rhinitis demonstrate enhanced mRNA levels of H1R and various pro-inflammatory cytokines, such as IL-4, IL-5, TNF-α, IFN-γ, etc. [84–87]. Pro-inflammatory mast cells and eosinophils also increase notably during the same condition [86]. Furthermore, the generation of mitochondrial ROS is responsible for inducing the IL-1β expression in allergic rhinitis [88]. iNOS expression was reportedly upregulated at the time of allergic rhinitis as well [89]. Therefore, the action of EPP against the allergic symptoms might be induced by antagonized H1R expression, suppression of pro-inflammatory mediators, including iNOS, ILs, and scavenging of various ROS/RNS. Besides, inflammatory cells in WBC, such as lymphocytes, neutrophils, eosinophils, monocytes, and basophils reduced in mice blood and BAL fluid, especially in the 500 mg/kg EPP treated group (Figs. 8A–F, 9A–F). Cytokines, such as IL-4, IL-5, IL-13, etc. are enhanced due to a rise in eosinophils, basophils, T$_H$2, and mast cells that fight the foreign allergens residing in blood [90]. In the bloodstream, lymphocytes, neutrophils, and monocytes also perform the same role as other WBCs to combat external allergic infections. Hence, a notable decrease in the number of different WBCs certifies the amelioration of allergic conditions [90]. As well, allergy induced via chemicals like TDI through the nasal airway greatly affects the bronchioles of the respiratory system that inevitably cause the rise of various WBCs and thereby upregulates the expressions of IL-4, IL-5, IgE, and T$_H$2 [91]. So, the anti-allergic role of EPP in mice BAL fluid might be accompanied by the downregulation of these cytokines, related to the decreased number of differential WBCs.

In-silico molecular docking simulation was carried out using the major metabolite of EPP, syringic acid against H1R and iNOS (Figs. 10A–D, 11A–D). While binding with both of these proteins, syringic acid formed a good number of H-bonds which is critical for molecules to obtain better binding affinities. In contrast with syringic acid, *trans*-ferulic acid and rosmarinic acid exhibited better binding affinities towards H1R and iNOS (S. Figs. S2–5) because of the availability of a higher number of –OH groups in their aromatic structures. Nevertheless, we propose that syringic is principally responsible for exerting the immune-modulatory functions of EPP. The reason behind this might be, though both *trans*-ferulic acid and rosmarinic acid exerted better binding interactions with H1R and iNOS, syringic acid is present in much higher amounts in EPP (89.19 mg in 100 g EPP). Therefore, the number of available –OH groups for exerting free radical scavenging property or better intermolecular interactions is expected to be higher in syringic acid. H1R is implicated in regulating several gene expressions, including p38 and NF-κB via increasing its binding with histamine [92]. Syringic acid being bound with H1R might antagonize this receptor and thus suppress the histamine levels that might also downregulate NF-κB.

As of now, there are 13 binding site agents for iNOS have been discovered in non-human primates, i.e., activator protein-1 (AP-1), NF-κB, etc. [93]. Hence, through binding with iNOS, syringic acid might abrogate the NF-κB activation, limiting its allergic inflammatory pathways. Considering the *in-vitro*, *in-vivo,* and *in-silico* evidences, EPP might be considered as a promising natural product that possesses significant potential against allergic inflammation. Despite the evidence presented in this article, it can't be denied that natural products always come up with a number of translational hurdles including, poor pharmacokinetic and oral bioavailability due to insolubility in water, low stability in gastric pH, rapid fast pass, microbial degradation in gut, etc. But recent researches on phytotherapy suggest that technologies like, polymeric nano-formulations, pro-drug approaches, efflux inhibitions, and permeation enhancements can be greatly handy in translating the phyto-constituents into drug-like substances to further proceed for clinical trial studies [94]. Considering the experiments conducted in this study, it is of no denying that several shortcomings are still present which are suggested to be addressed for future studies like this. Macrophage is an useful biomarker to understand the degree of inflammation [95]. When it comes to analyzing BAL fluid, macrophages could be counted along with other inflammatory cells to establish the role of EPP in exerting the targeted action. In addition, histopathological analysis of mice lung is suggested to be conducted in studies like this to further confirm the role of the experimented natural product against allergic inflammation.

5. Conclusion

This study reported for the first time the role of syringic acid-rich EPP in attenuating allergic inflammation. The *in-vitro* assays suggested that EPP strongly suppressed NO and ROS/RNS production. The iNOS, COX-2, NF-κB, IL-6, and TNF- α signaling were significantly downregulated by EPP. This role of EPP was later supported by the *in-vivo* studies conducted on TDI-induced allergic and formaldehyde-induced inflammatory animal models. The safe use of EPP in animals was evidenced by both acute and sub-acute toxicity studies. The *In-silico* study showed satisfactory binding affinities against both H1R and iNOS which further backed up the promising potential of EPP in allergic inflammation. This study suggests further research on syringic acid and its role in ameliorating chronic and acute allergic inflammatory diseases. Especially, dietary application of syringic acid in varied allergic conditions might be targeted along with the existing treatment options to reduce the disease burden.

Author contribution statement

Md. Arman Islam: Performed the experiments, Analyzed and interpreted the data, Wrote the paper. Md Samiul Huq Atanu: Performed the experiments, Analyzed and interpreted the data, Contributed reagents, materials, analysis tools or data. Md Afjalus Siraj: Conceived and designed the experiments, Analyzed and interpreted the data, Wrote the paper. Rabindra Nath Acharyya: Performed the experiments, Analyzed and interpreted the data. Khondoker Shahin Ahmed: Performed the experiments, Analyzed and interpreted the data. Shrabanti Dev: Conceived and designed the experiments, Contributed reagents, materials, analysis tools or data, Wrote the paper. Shaikh Jamal Uddin: Analyzed and interpreted the data, Wrote the paper. Asish Kumar Das: Conceived and designed the experiments, Contributed reagents, materials, analysis tools or data, Wrote the paper.

Data availability statement

Data will be made available on request.

Additional information

Supplementary content related to this article has been given.

Declaration of competing interest

The authors have declared that they have no competing financial interest or personal relationship that could appear to influence the work outlined in this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e13343.

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