

Evaluation of Anti-Asthmatic Activity of Caffeic Acid in Allergen Induced Murine Allergic Asthma Model

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Abstract: *Considering the lack of effective drug to treat allergic asthma and based on the possible anti-allergic effect of caffeic acid (CA), this study was conducted to investigate the effects of caffeic acid in an experimental model of respiratory allergy to ovalbumin in adult balb/c mice, in order to further explore its possible effect as anti-allergic agent for the treatment of allergic asthma. Natural phenolic compounds are commonly present in considerable portions of the human diet. Caffeic acid (CA) is one of the major representative hydroxycinnamic acids widely distributed in plant tissues, present in foods such as fruits, spices, vegetables, wine, olive oil, and coffee. In-silico docking studies of caffeic acid with TNF-alpha receptor structure was performed using Glide version 9.6 of Schrödinger software. Antiasthmatic activity was studied using in-vivo mice BALF and lung homogenate studies and in-vitro TNF-alpha inhibition method.*

Keywords: Caffeic acid, Ovalbumin, Allergic Asthma, TNF-alpha.

1. Introduction

Asthma, a disease of airways occurs in people of all ages. The most revised global estimate of asthma suggests that as many as 334 million people have asthma and that the burden of disability is high [1]. The structural changes in the airway including subepithelial and airway wall fibrosis, goblet cell hyperplasia/metaplasia, smooth muscle thickening and increased vascularity are observed [2]. The complex interactions between immunological mediators that are produced by inflammatory cells, such as T-lymphocytes, mast cells, macrophages, eosinophils, basophils, neutrophils, dendritic cells (DCs), and structural cells elicits to the main pathological features of allergic asthma [3]. As a result, many structural changes occur in the cellular and extracellular parts of the asthmatic bronchi.

Mediators released by mast cells are categorized into preformed mediators, for example, prostaglandins (PGs) and leukotrienes (LTs), and cytokines like tumor necrosis factor alpha (TNF- α) [4]. These mediators cause the symptoms of immediate-type hypersensitivity that may develop mild rhinitis to serious conditions like anaphylactic shock. The immediate symptoms in the respiratory tract include coughing, bronchospasm, smooth muscle constriction, edema, mucus secretion, and eventually causing the formation of an inflammatory infiltrate [5], [6]. Granulocyte macrophage-colony stimulating factor (GM-CSF), cytokines (TNF-alpha, IL-3 and IL-4) and chemokines (especially CCL11 (eotaxin-1), but also CCL3 (MIP-1a), CCL5 (regulated upon activation, normal T-cell expressed, and secreted (RANTES), CCL7 (monocyte chemoattractant protein (MCP)-3), CCL22 (macrophage-derived chemokine, MDC)) do play an important role in eosinophil differentiation, proliferation and recruitment [7], [8].

Corticosteroids, β_2 -adrenergic agonists, phosphodiesterase (PDE) inhibitors and leukotriene receptor antagonists are the most common drugs used to treat asthma and other allergic respiratory diseases. These agents are able to control the

illness symptoms but also induce long-term adverse effects, such as osteoporosis, growth stunting in children, hyperglycemia, and hypokalemia [9]. Hence, alternative treatments are imminent to improve patient adherence and disease control. Caffeic acid (CA) is one of the major representative hydroxycinnamic acids. Some biological activities of CA have been reported in the literature, such as anti-oxidant [10], anti-colitic, antinociceptive and analgesic [11]. Interestingly, the CA also seems to have anti-inflammatory effects due to the suppression of pro-inflammatory cytokines production such as IL-1b and IL-6, and also through the inhibition of vascular permeability, possibly, by an inhibition of histamine release from mast cells and leukotriene biosynthesis inhibition. Others also mentioned that caffeic acid may subdue the activation of transcription factors such as NF-kB and inhibit the intracellular signaling cascades such as mitogen-activated protein kinase (MAPK) [12]. Caffeic acid has shown a wide range of potential pharmacological effects both in in-vitro studies and in animal models. The inhibitory effect of caffeic acid in human HT-1080 fibrosarcoma cell line has recently been established [13] as it inhibits cancer cell proliferation by an oxidative mechanism. Caffeic acid is an antioxidant in vitro and also in vivo [14]. CA showed immunomodulatory and anti-inflammatory activity [15]. There have been no known ill-effects of caffeic acid in humans apart from being a thiamine antagonist (anti-thiamine factor).

2. Materials and Methods

2.1 Chemicals

Ovalbumin from LobaChem, O-dianisidine from Koma Biotech, NEDD from Fisher Scientific, Tumor Necrosis Factor - α Kit from Koma Biotech.

2.2 Methods

2.2.1 In-silicostudies

In-silico study involved docking studies of TNF- α receptor protein with caffeic acid targeting mouse 2TNF receptor using Glide/Schrodinger software. Caffeic acid was prepared using LigPreP.

2.2.2 In-vivo studies

2.2.2. (a) Animals

Adult Balb/ mice of either sex of 50 days (20-28g) were obtained from the Central Animal House, JSS College of Pharmacy, Ootacamund. The animals were housed under laboratory conditions (relative humidity 35 \pm 2%, temperature 22 \pm 2 $^{\circ}$ C and 12h light and dark cycle). They were fed with standard rodent pellet diet and purified water ad libitum. The study was approved by the institutional animal ethics committee for animal care and use). The animals were approved by IAEC (Institutional Animal Ethical Committee

Table 1: Grouping of animals.

Groups	Treatment
I	Normal control (0.9g of NaCl in 100ml of water)
II	Negative control (Ovalbumin-sensitized)
III	Positive control (Ovalbumin-sensitized + Dexamethasone 3mg/kg)
VI	Ovalbumin-sensitized + Caffeic acid 50mg/kg ⁻¹
VII	Ovalbumin-sensitized + Caffeic acid 100mg/kg ⁻¹

2.2.2. (b) Procedure

A preparation of Ovalbumin was prepared as per previously described method [16]. Briefly 80 mg of ovalbumin was dissolved in a 40 ml cold sterile saline and vortexed for 5 min at 2,000 rpm to mix and distribute 30 ml of the solution. The resulting ovalbumin aliquots were immediately store frozen at -20 $^{\circ}$ C for up to 2 months.

Caffeic acid was freshly prepared in 50mg/ml and 100mg/ml concentrations in canola oil and was administered by gavage once a day for 7 days. Induction of allergic asthma in Balb/C mice using ovalbumin as an allergen.

Allergic asthma was induced in adult Balb/C mice using ovalbumin as an allergen in three steps according to a previously described method [17] which included:

1. Allergen sensitization using a freshly prepared suspension containing 0.5 mg/ml of ovalbumin and 20 mg/ml of alum in sterile saline (0.9% NaCl). "1 ml" syringes and "25G" needles were used to inject i.p. 100 μ l suspension per mouse. The suspension was gently homogenized by 3-4 repeated reversals of the tube/syringe before administration (so that the suspension does not drop at the bottom of the tube/syringe).

2. Allergen challenge: Mice were challenged on the 14th and 21st day after sensitization using saline aerosol for control mice and 2.5 % w/v ovalbumin aerosol for other groups, both delivered through a nebulizer (Smart care TM Ultrasonic Nebulizer, WH 2000) for 20 minutes at 30-36 psi pressure. The particle size ranged from 0.5 -5 μ .

3. Treatment included administration of caffeic acid or normal saline orally from day 14 to 21, and also an hour

before ovalbumin aerosol challenge during 14th and 21st days.

On the 21st day, after 24h of the last challenge mice were sacrificed by cervical dislocation to collect bronchoalveolar lavage fluid. The trachea was cannulated and BALF obtained by washing the airway lumina by inserting tracheal cannula via mid cervical incision and lavaged twice with 1ml of ice cold phosphate buffered saline (PBS, pH=7.4). After collecting bronchoalveolar lavage fluid (BALF), the lungs of mice were removed and a part of it was stored at -20 $^{\circ}$ C for the estimation of eosinophil peroxidase (EPO), myeloperoxidase (MPO) and malondialdehyde (MDA) levels.

2.2.2. (c) Total and differential cell count.

The BALF was centrifuged at 170g for 10min at 4 $^{\circ}$ C and the supernatant was removed and stored at -80 $^{\circ}$ C for the estimation of nitrite and nitrates. The pellets obtained after the centrifugation were resuspended in 0.5ml of the PBS and total leukocyte count was performed using Neubauer's chamber and WBC diluting fluid.

Total cells (10⁴ /ml) = $x \cdot 0.4 \times F \times 1000$,

where, X = total cells counted in 4 squares and F = dilution factor

The air dried smeared slide was stained for 10min with Giemsa and washed with distilled water for 8min. Counterstaining was later carried out with May-Grunwald stain for 10 minutes. The differential cell count was carried out using a digital light microscope at 100x magnification by oil immersion technique (Motic, Japan, Cat. No. B1 Series). At least 200 cells were distinguished on each slide [18].

2.2.2. (d) Measurement of Eosinophil Peroxidase

Suspension of BALF and pulmonary homogenates were freeze-thawed 3 times using liquid nitrogen and a water bath at 37 $^{\circ}$ C to obtain the EPO. The fluid was centrifuged at 1000G at 4 $^{\circ}$ C for 10 minutes. 75 μ l of the supernatant was serially diluted in a 96 well plate followed by the addition of 150 μ l substrate. A substrate was prepared using 1.5mM O-Phenyl diamine and 6.6mM Hydrogen peroxide in 0.05M Tris-HCl, pH 8.0. After incubation at room temperature for 30 minutes' reaction was stopped by addition of 75 μ l of 30% sulphuric acid. The absorbance of a sample was determined at 492 on ELISA reader. [19]

2.2.2.4 Estimation of lung tissue myeloperoxidase (MPO) concentration

The frozen isolated lungs were weighed and tissue was minced and homogenized with 1 ml of the 50 mM phosphate buffer (pH=6) using Teflon coated glass high-speed homogenizer. The homogenate was centrifuged at 40000 g at 4 $^{\circ}$ C for 15 min. The pellets were resuspended in 1ml 50 mM potassium phosphate buffer (pH=6) containing 0.5 % (w/v) hexadecyltrimethylammonium bromide (HTAB) to neutralize the pseudo-peroxidase activity of haemoglobin and to solubilize membrane bound MPO. The suspension was frozen and thawed three times and ultra-sonicated to release cell contents. 0.1 ml of the sample was added with 2.9 ml of potassium phosphate buffer (50mM) containing O-dianisidine (0.19 mg/ml). The solution was transferred to the cuvette and hydrogen peroxide [0.0005 % (v/v)] was added.

Immediately the absorbance was measured at 460 nm and after 3 min using UV spectrophotometer. The MPO activity per gram wet lung was calculated [20], [21].

2.2.2. (e) Measurement of malondialdehyde (MDA) level in lung tissue

The lung tissue (about 100 mg) was homogenized with 1 ml normal saline using Teflon coated glass high-speed homogenizer. 0.5M 0.2ml Tris-HCl was added to 0.2ml tissue supernatant and incubate at room temperature for 2 hours in a 2ml Eppendorf tube. Add 0.4 ml of 10% Tri Chloroacetic acid (TCA) was added and centrifuged at 1000G for 10 minutes. To 0.1 ml of the supernatant obtained, add 0.4ml of 0.67% thiobarbituric acid (TBA) and the tubes were kept in boiling water bath for 10 minutes. After cooling the solution add 0.4ml double distilled water. Check the absorbance of the pink solution at 532nm in a microplate reader. The extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ is used and the value was expressed as nmol of MDA/mg of protein [22].

2.2.2. (f) Nitrite determination using Griess reagent

Nitric oxide (NO), a significant molecular mediator for many physiological processes, such as vasodilation, inflammation, thrombosis, neurotransmission, and immunity is a prominent agent in inflammation. There exist lots of methods to measure NO in biological systems. One of these methods involves the use of the Griess diazotization reaction to spectrophotometrically detect nitrite formed by the spontaneous oxidation of NO under physiological conditions as per a previously described method [23] – [25].

2.2.2. (g) Estimation of TNF- α in BALF collected from mice

The protocol for TNF- α estimation according to the manufacturer’s instructions is as follows: 200ul of washing solution was added to each well. The wells were aspirated to remove liquid and the plate was washed 3 times using 300ul of washing solution per well. After the last wash, the residual solution was removed by inverting the plate and by blotting on a paper towel. 100ul of standards or sample was added to each well in duplicate and covered with the Plate Sealer provided. This reaction mixture was incubated for a span of 2 hours at room temperature. The plate was then aspirated to remove liquid and washed 4 times. Later development enzyme (1/200) per well was added and covered with the plate sealer provided. After 30 minutes of incubation at room temperature the plate was aspirated and washed 4 times as in step 1. 100ul of color development solution was added to each well and incubated for 20-40 minutes until proper color development. To stop the colour reaction, 100 μ l of the stop solution was added to each well. To stop the color reaction, 100ul of the stop solution was added to each well. Using a microtiter plate reader, the readings were taken at 450nm wavelength.

2.2.2. (h) Histopathology of lung tissue

The lung tissue was stored in neutral buffer 10 % formalin. The paraffin embedded blocks were cut into 6 μ m section using a microtome (Lecia, UK; Model No.RM2135), mounted and stained with hematoxylin and eosin stain for routine histology.

3. Results and Discussions

3.1 Docking score of caffeic acid with mouse 2TNF, TNF- α receptor.

2TNF is a 1.4 A resolution structure of recombinant mouse tumor necrosis factor alpha (mTNF). The mTNF and human TNF are resembling in structure, only diverging mainly in regions that are either flexible and/or involved in crystal packing.

Table 2: Ligand interaction scores of caffeic acid with mTNF receptor

Ligand	GScore	Lipophilic EvdW	PhobEn	HBond	Electro
Caffeic acid	-4.91	-1.77	-0.25	-1.12	-1.77
IA	-2.13	-0.98	-1.21	-1.33	-0.42

Abbreviations: IA (Isopropyl alcohol), GScore (Glide score), PhobEn (hydrophobic enclosure reward), HBond (Hydrogen bond), Electro (Electrostatic reward), vdW (Van der Waals energy)

The results revealed caffeic acid’s interaction through hydrogen bonding with 2 molecules of amino acid TYR 119 with a docking score of -4.91 which is compared with IPA. One 2-propanol(IPA) molecule trapped in the trimeric channel is a lead compound for the design of TNF inhibitors. Caffeic acid also got trapped in the trimeric channel with better affinity than IPA through hydrogen bonding.

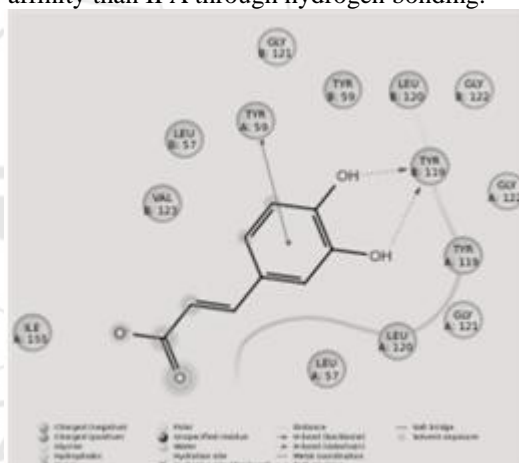


Figure 1: Ligand Interaction Diagram of caffeic acid with 2TNF TNF- α receptor

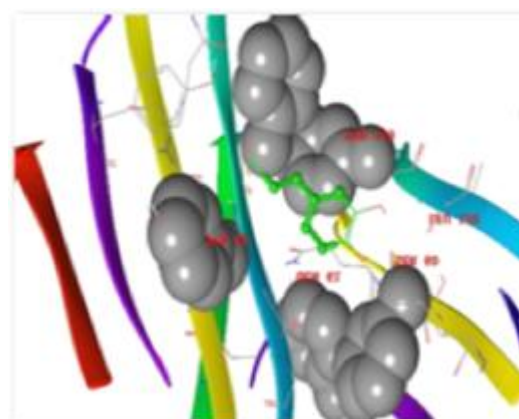


Figure 2: Hydrophobic interactions of caffeic acid with 2AZ5 TNF- α receptor

3.2 Effect of caffeic acid on total and differential cell count

Caffeic acid significantly reduced the total cells (both at 100 mg/kg and 50 mg/kg), macrophages, eosinophils, lymphocytes and neutrophils in BALF. The results show anti-inflammatory property of caffeic acid in terms of reduction of the inflammatory cells (lymphocytes and neutrophils).

Table 3: Differential (Neutrophil, monocyte and macrophage) cell count

S. no	Treatment	Percentage (%) of		
		Neutrophil count	Monocyte count	Macrophage count
1	OVA Negative control	40.02±1.551###	7.955±0.3347#	63.23±1.025###
2	Control	25.35±2.800	1.861±0.0939	55.68±3.002
3	Dexa 3mg/kg (positive control)	31.06±1.53***	2.832±0.1469*	43.51±1.24863**
4	CA 50mg/kg	31.57±0.5738*	4.089 ± 0.2046***	56.09±1.065*
5	CA 100mg/kg	29.08±2.016**	3.059±0.1971*	50.37±0.9097**

There is a significant increase in total cells in OVA control (109±1.732) (P< 0.0001) when compared to normal control (54±2.464). This increase is indicative of inflammation in BALF. This shows Ovalbumin was able to induce allergic inflammation in animals.

Table 4: Total and differential (Eosinophil and lymphocyte count) cell count

Sl.no	Treatment	Total cells count (*10 ⁴)	Percentage (%) of	
			Eosinophil Count	Lymphocyte count
1	OVA Negative control	109 ±1.732###	16.8 ± 0.5447###	5.516 ± 0.5874#
2	Control	54 ± 2.464	2.133 ± 0.7344	2.436 ± 0.5103
3	Dexa 3mg/kg (positive control)	64.33 ± 0.6667***	2.58 ± 0.4956***	2.289 ± 0.1656**
4	CA 50mg/kg	87 ± 4.359***	7.757 ± 1.221***	3.622 ± 0.4402*
5	CA 100 mg/kg	72 ± 1.528***	5.592 ± 0.9075***	3.132 ± 0.3072**

Values expressed as Mean ± SEM, n = 6, Statistical significance: ### P<0.001 vs. control: * P<0.05, ** P<0.01, *** P<0.001 vs. negative control; One Way ANOVA followed by Dunnett's multiple comparison test. Route of administration: Oral.

3.3 Caffeic acid reduces eosinophil peroxidase (EPO) in BALF, myeloperoxidase (MPO) and malondialdehyde (MDA) levels in lung tissue

EPO is found in cytoplasmic granules of human eosinophilic leukocytes and is much less active than MPO in Cl⁻ oxidation but is much more active with Br⁻, I⁻, and SCN⁻.

These play a significant part in the loss of membrane integrity, structural or functional alterations in proteins and genetic mutations, by interacting with lipids, proteins and nucleic acids respectively. The results reveal a significant (P<0.001) decrease in EPO levels (2.072±0.1298) and (2.25±0.2304); (P<0.01) when treated with 50 mgkg⁻¹, 100 mgkg⁻¹, dose levels of caffeic acid respectively when compared to ovalbumin sensitized group (3.547±0.1465). Caffeic acid is effective in decreasing EPO levels in lung tissue homogenate. Whereas dexamethasone (3mg/kg) showed a significant decrease (1.912±0.2805); (P<0.001) and was able to bring the EPO levels close to normal control (0.8869±0.0279). Dexamethasone modulates negative cell migration into sites of inflammation and this action is accomplished, in part, by a potent inhibitory effect on the synthesis of proinflammatory cytokines such as IL-4, TNF-α, and chemokines coupled to a reduced degree of leukocyte responsiveness.

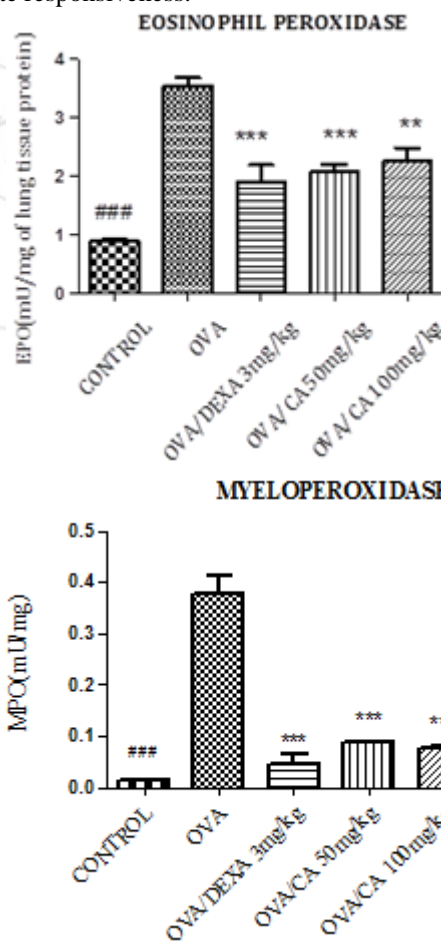


Figure 3: Effect of treatment with caffeic acid (CA) on eosinophil peroxidase and malonyldialdehyde levels in ovalbumin-induced mice model.

Lung tissue myeloperoxidase (MPO) concentration reveals the level of cell damage in the lungs. Neutrophils release Myeloperoxidase (MPO) in response to invading pathogens. Being one of the earliest lines of defense in innate immunity MPO acts by catalyzing the hydrogen peroxide-mediated oxidation of halide ions to products such as hypochlorous acid (HOCl). The results reveal a significant (P<0.001) decrease in myeloperoxidase levels (0.08668±0.0039) and (0.0768±0.005) when treated with 50 mgkg⁻¹, 100 mgkg⁻¹, dose levels of Caffeic acid respectively when compared to ovalbumin sensitized group (0.3795±0.036). Caffeic acid at

both doses was able to inhibit lung cell damage. Whereas dexamethasone (3mg/kg) showed significant decrease (0.0463 ± 0.0182); ($P < 0.001$). Dexamethasone downregulates NF κ -B gene transcription thereby reducing neutrophil adhesion and migration.

Estimation of malondialdehyde(MDA) benefits in recognizing the degree of lipid peroxidation in the lung tissue. The results reveal a significant ($P < 0.001$) decrease in malondialdehyde levels (10.5 ± 4.908) and (3.267 ± 0.7535) when treated with 50 mgkg⁻¹, 100 mgkg⁻¹, dose levels of Caffeic acid respectively when compared to ovalbumin sensitized group (27.77 ± 0.9905) and close to control (1.355 ± 0.2139). Because MDA is described as an oxidative marker, the results of this study indicate caffeic acid reduced the oxidative stress in cells. Whereas dexamethasone (3mg/kg) showed asignificant decrease (7.267 ± 2.339); ($P < 0.001$)when compared to ovalbumin sensitized group (27.77 ± 0.9905).

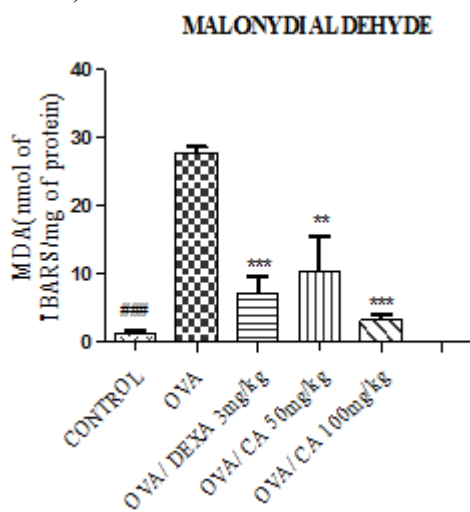


Figure 4: Effect of treatment with caffeic acid (CA) on Malonyldialdehyde levels in ovalbumin-induced mice model

3.4 Caffeic acid substantially reduced Nitrite levels

Increased levels of nitrite and nitrate in lungs lead to the formation of NO which causes oxidative stress. The combined effect of elevated oxidative stress and nitric oxide may lead to the formation of the potent peroxynitrite radical which could result in nitrosylation of proteins in the airways. The results reveal a significant ($P < 0.01$) decrease in nitrite levels as (23.4 ± 0.7676) and ($P < 0.001$), (18.61 ± 1.125) when treated with 50 mgkg⁻¹, 100 mgkg⁻¹, dose levels of caffeic acid respectively when compared to ovalbumin sensitized group. Caffeic is effective in decreasing nitrite levels in BALF. Whereas dexamethasone (3mg/kg) showed asignificant decrease (22.93 ± 1.541); ($P < 0.01$) and was able to bring the nitrite levels close to normal control (16.74 ± 0.602). Dexamethasone acts by the inhibition of inducible nitric oxide synthase (iNOS), by a number of factors involved in inflammation, including interleukins, interferon- γ .

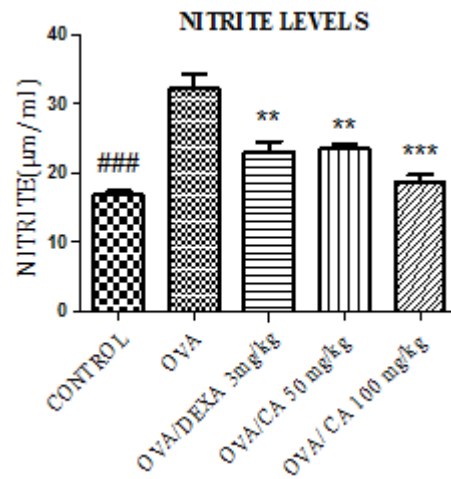


Figure 3: Effect of treatment with caffeic acid (CA) on nitrite levels in ovalbumin-induced mice model.

3.5 Treatment of caffeic acid reduces the amount of TNF- α in half collected from balb/c mice

TNF- α is an amplifying mediator in asthma and is produced in increased amounts in asthmatic airways. TNF α promotes recruitment of neutrophils, as well as eosinophils, into the airways through functional effects on the endothelium and by direct and indirect chemotactic effects. The results reveal a significant ($P < 0.01$) decrease in TNF- α levels (85.07 ± 0.1422) and ($P < 0.05$) (105.2 ± 0.1831) when treated with 50 mgkg⁻¹, 100 mgkg⁻¹, dose levels of caffeic acid respectively when compared with ovalbumin sensitized group. Caffeic is effective in decreasing TNF- α levels in BALF. OVA negative control group shows high TNF- α levels which are characteristic of inflammation. There was no significant difference in TNF- α levels between (85.07 ± 0.1422) 50 mgkg⁻¹ and (105.2 ± 0.1831) 100 mgkg⁻¹ dose levels of caffeic acid. Lower dose produced more inhibition of TNF- α than the higher dose, so lower dose of caffeic acid could be used effectively in future. Caffeic acid 50mg/kg was able to produce a significant effect, so this dose can be preferred over caffeic acid 100mg/kg in future. Whereas dexamethasone (3mg/kg) showed a significant decrease (58.89 ± 0.1013); ($P < 0.001$) when compared to ovalbumin sensitized (159.8 ± 0.0517). Standard drug was able to bring the levels close to normal control (46.69 ± 0.0329).

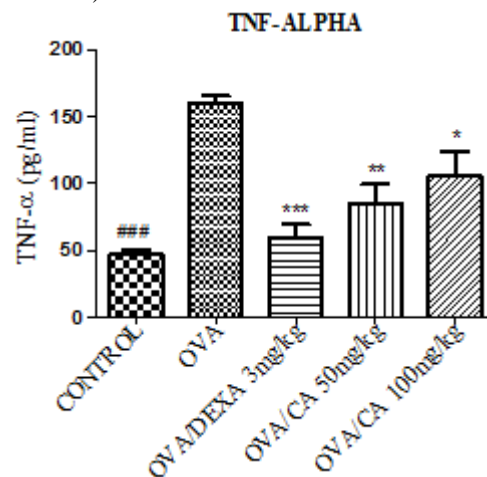


Figure 3: Statistical analysis of TNF- α levels. Values expressed as Mean \pm SEM, n = 6, Statistical significance:

$P < 0.0003$ vs. control: ***; One Way ANOVA followed by Dunnett's multiple comparison tests. Route of administration: Oral

3.6 Treatment with caffeic acid ameliorates Ovalbumin-induced pathological changes

Modifications of lung histology are illustrated in (Fig A–D). In the vehicle control mice, the airway and peribronchovascular space were essentially normal (Fig B). Caffeic acid 50mg/kg and 100mg/kg showed a reduction in goblet cells presence and musculature (Fig C and D) comparable to that of control (Fig 26A).

Standard drug Dexamethasone 3mg/kg significantly decreased both lung parenchyma tissue thickness (Fig B). Dexamethasone inhibits allergen-induced eosinophilia and airway hyperresponsiveness through down-regulation of pro-inflammatory cytokines such as IL-10, TNF- α .

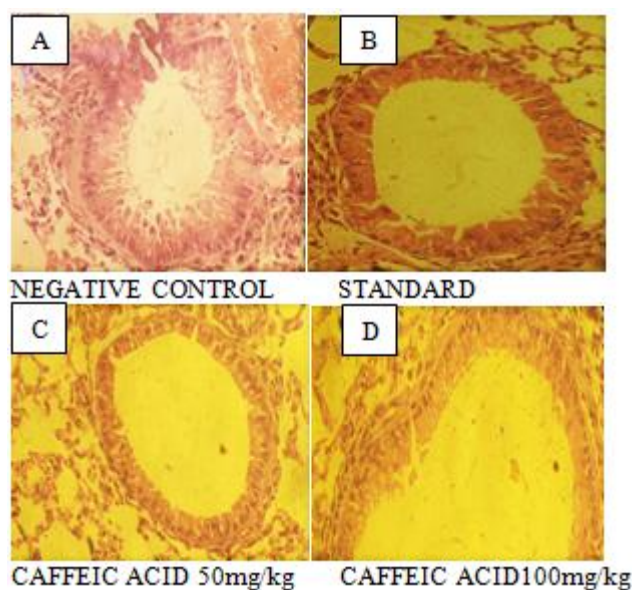


Figure 3: Modifications of lung histology with the help of haematoxylin and eosin stain are illustrated in (Fig A–D). A) Histopathology features of vehicle treated OVA-sensitized mice lung
B) Histopathology features of Dexamethasone 3 mg/kg treated OVA-sensitized mice lung.
C) Histopathology features of Caffeic acid 50mg/kg treated OVA-sensitized mice lung.
D) Histopathology features of Caffeic acid 100mg/kg treated OVA-sensitized mice lung.

4. Conclusion

The immune-mediated disorder, asthma is defined by marked airway hyperresponsiveness, eosinophilic airway inflammation, and increased mucus production. OVA-exposed mice expressed the three main characteristics of human asthma, namely, BHR, eosinophilic airway inflammation, and airway remodeling, characterized by subepithelial collagen deposition, airway fibrosis, and increased BSM mass [15]. In the present study, caffeic acid, a phenolic acid found in fruits and vegetables, significantly reduced the total inflammatory cells and the number of eosinophils, EPO activity, myeloperoxidase activity, MDA

activity and the levels of nitrite and TNF α in BALF. These effects may be attributed to its inhibitory effect on calcium-activated potassium channel KCa3.1. Heike Wulff et.al., reported that in cells such as erythrocytes, activated T and B cells, macrophages, microglia, vascular endothelium, epithelial, and proliferating vascular smooth muscle cells, membrane potential and calcium signaling is regulated by calcium-activated potassium channel KCa3.1 [26]. Aida Oliva'n-Viguera et.al., identified caffeic acid and resveratrol as the first natural phenolic phytochemicals with a remarkable KCa3.1-blocking efficacy as indicated by EC50s in the lower micromolar range (1–10 μ M) [27]. The KCa3.1 inhibitor Senicapoc has been shown to reduce the allergen-induced increase in airway resistance in allergic asthma patients, but not the asthmatic responses to exercise challenge in patients with mild asthma. [28]

Pierre-Olivier Girodet et.al., demonstrated that curative treatment with the specific KCa3.1 blocker TRAM-34 abolishes BSM remodeling and sub-basement collagen deposition, and attenuates airway eosinophilia in a murine model of chronic asthma [29]. Blocking KCa3.1 has great potential in the treatment of chronic asthma by preventing the increased mass of bronchial smooth muscle associated with airway remodeling. In contrast, Dexamethasone at a cellular level, reduce the number of inflammatory cells in the airways, including eosinophils, T lymphocytes, mast cells, and dendritic cells [30]. This is one of the reasons why scientists believe that corticosteroids act by inhibiting the effects of pro-inflammatory transcription factors, such as AP-1 and NF- κ B, regulate gene expression and affects many inflammatory proteins synthesis including cytokines, inflammatory enzymes, adhesion molecules, and inflammatory receptors [31]. In the present study, Dexamethasone showed anti-inflammatory property by decreasing inflammatory cell counts such as eosinophils, neutrophils and reduced TNF- α level in BALF. Furthermore, in future, it would be interesting to study the activity of caffeic acid in TNF α /IFN γ -associated glucocorticoid insensitivity studies using cultured airway smooth muscle cells. Other KCa3.1 channel blockers like TRAM-34, ICA-17043, were effective in inhibiting GC-resistant CCL5, CCL11 and CX3CL1 in ASM cells [26]. Fluticasone with KCa3.1 blockers or KCa3.1 downregulation was effective in inhibiting GC-resistant CCL5, CCL11, and CX3CL1 in ASM cells [32].

5. Acknowledgement

The Authors are grateful to JSS College of Pharmacy, JSS University, Ooty, Tamil Nadu, for providing necessary facilities to carry out this work

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