

Capsazepine, rimonabant, WIN55, 212-2 and lidocaine attenuated acute lung inflammation induced by co-exposure of capsaicin and cigarette smoke extract in rats

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Cigarette smoking is central to the pathogenesis of lung inflammatory diseases like asthma and chronic obstructive pulmonary disease (COPD). The study evaluated the effect of drugs belonging to pharmacologically different classes *viz.*, capsazepine, a TRPV1 antagonist; rimonabant, a CB1 antagonist; WIN 55,212-2, a cannabimimetic; and lidocaine, a local anaesthetic in lung inflammation induced by cigarette smoke extract (CSE) and Capsaicin. Capsazepine (10 mg/kg), rimonabant (3 mg/kg), WIN 55,212-2 (3 mg/kg), and lidocaine (1 mg/kg), were intraperitoneally (i.p.) administered to Wistar rats. They were then exposed to capsaicin (20 mg of *Capsicum oleoresin*/kg body weight i.p.) followed by intratracheal administration of CSE (1.3 mL/kg). After 24 hours, Bronchoalveolar lavage (BAL) was performed, and lungs were removed and processed to assess the various lung inflammatory parameters. Co-exposure with capsaicin and CSE lead to rise in leucocyte counts and total proteins in bronchoalveolar lavage fluid (BALF). Pretreatment with capsazepine, rimonabant, WIN 55,212-2 and lidocaine significantly abrogated the lung inflammation. They also prevented the rise in lung tumor necrosis factor α (TNF α), myeloperoxidase (MPO) and matrix metalloproteinase 9 (MMP 9) activities. This was further corroborated with histopathological evidences. The study reveals that these drugs act through distinct mechanisms to abate capsaicin- and CSE-induced lung inflammation in rats. The effects may be attributed to direct or indirect inhibition of the inflammatory cascade after transient receptor potential vanilloid (TRPV1) channel activation by CSE and capsaicin. The impact of the test drugs in reducing capsaicin plus CSE induced lung inflammation makes them potential candidates for the treatment of lung inflammatory diseases.

Keywords: Asthma, Cannabimimetic, *Capsicum oleoresin*, Chronic obstructive pulmonary disease (COPD), Matrix metalloproteinase 9 (MMP 9) activity, Myeloperoxidase (MPO), Smoking, Tumor necrosis factor α (TNF α)

Lung diseases continue to be one of the leading causes of death across the globe. Bronchodilators, leukotriene antagonists, mast cell stabilizers, phosphodiesterase inhibitors, glucocorticoids and non-steroidal anti-inflammatory drugs (NSAIDs) are the bastion of current management for patients with lung inflammatory diseases like asthma and COPD¹. These treatments are often disappointing and cannot stop the progression of these diseases, thus, prove to be poorly reversible. The inflammatory processes involved are complex². It is reported that neutrophil chemotactic mediators such as interleukin-8 and leukotriene B4, and proinflammatory cytokines, such as tumor necrosis factor α (TNF α) are increased in the sputum of patients with COPD, as compared with that of normal subjects³.

Cigarette smoking, both active and passive, is identified as an important causative factor in the

development of lung inflammatory diseases. Although the role of cigarette smoking in asthma is not clear, it may act as a trigger or as an exacerbating factor for asthma⁴. Pulmonary neutrophil influx is a common feature of the chronic inflammatory lung diseases⁵. The tobacco-induced necrosis exacerbated release of neutrophil-derived proteolytic enzymes, particularly elastase and matrix metalloproteinases (MMP)^{8,9} have been proposed as a key event in development of destructive lung diseases⁶. Intratracheal instillation of cigarette smoke and/or its extract has been employed as an inducing agent for airway inflammation in rodents and researchers have been using it in different mediums to study the deleterious effects of cigarettes both *in vitro* and *in vivo*.^{4,7-9} Accordingly, in this study, CSE and a known lung irritant capsaicin found in hot chilli peppers were employed to induce lung inflammation in rats. It is well established that the vanilloid, capsaicin activates specific member of the ligand-gated ion channel family, TRPV1. CSE is also known to act on the TRPV1 receptors¹⁰. It was, thus,

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hypothesized in this study that acute exposure to CSE could potentiate the inflammatory response to capsaicin. The expression of TRPV1 channels in the mammalian respiratory tract is predominantly localized in non-myelinated C fibres. Activation of TRPV1 channels on airway sensory nerves induces calcium (Ca^{2+}) influx into the neuronal cell resulting in extensive reflex responses such as bronchoconstriction, mucus secretion, bradycardia and hypotension¹¹. Together with TRPV1, the capsaicin receptor, TRPA1 is also known to contribute to chronic cough, and airway inflammation in asthma and COPD¹². Patients with lung inflammatory diseases are more sensitive to the tussive effect of TRPV1 agonists while TRPV1 activation may be responsible for some of the respiratory symptoms in the airways during asthma exacerbation or in other conditions like COPD¹³. In the view of above facts, TRPV1 antagonists were deemed to have potential use in the therapy of these diseases.

In this context, we evaluated four drugs, of which other than capsazepine, the rest three are conventionally not known to act as TRPV1 antagonists but recent reports suggest their action via TRPV1 modulation¹⁴⁻¹⁸. We explored the impact of capsazepine (TRPV1 antagonist), rimonabant (CB1 antagonist), WIN 55,212-2 (cannabimimetic) and lidocaine (local anaesthetic) on lung inflammation induced in Wistar rats by intraperitoneal capsaicin exposure followed by intratracheal CSE instillation. Micro RNAs (miRNAs) are one of the most important, evolutionarily conserved, non-coding RNA molecules that play a crucial role in gene expression. They are transcribed as long primary transcript (pri-miRNA), which comprises several kilo bases, and then are processed into a smaller (~70 nts) precursor (pre-miRNA) by the microprocessor complex. This hairpin intermediate (pre-miRNA) is then transported to cytoplasm via exportin 5-Ran GTP complex, and is further processed by dicer enzyme, producing the mature duplex miRNA. The guide strand gets incorporated into RNA-induced silencing complex (RISC), followed by binding to the 3'-UTR (untranslated region) of their target mRNAs and regulates post-transcriptional gene expression via translational inhibition and/or mRNA decay¹.

Materials and Methods

Cigarettes of common Indian brand (WILLS Navycut®) were purchased from a local Indian shop.

Capsaicin was obtained as a gift sample from Omniactive Health Technologies (Mumbai, India). Capsazepine was purchased from Tocris Bioscience (Missouri, USA). Rimonabant, (R)-(+)-WIN 55,212-2 mesylate salt and O-dianisidine dihydrochloride were purchased from Sigma-Aldrich (St Louis, MO, USA). Lidocaine hydrochloride was obtained as a gift sample from Gufic Biosciences Ltd. (Mumbai, India). Lidocaine hydrochloride solution was prepared in saline. Giemsa stain was obtained from Qualigens fine chemicals (India).

Preparation and standardization of CSE

Methodologies as described in earlier works were adapted^{17,19,20}. In brief, a special apparatus was designed for preparation of cigarette smoke extract (CSE). The apparatus was connected to the vacuum pump. Filter tipped cigarette was connected to a cigarette holder attached to the apparatus. The cigarette was lit at the open end and the airflow was adjusted in such a way that 1 cigarette took 1 min for complete combustion. CSE was prepared by bubbling smoke from four cigarettes into 10 mL PBS at a rate of one cigarette per minute. CSE was prepared just 20 min before administration to animals and stored at 4°C in airtight glass bottles. Differences in CSE content from one extract to another were checked for its efficacy by its absorbance at 524 nm (0.6675 ± 0.01). Freshly prepared CSE was administered to animal groups from same preparation on the same day. For control group, air bubbled PBS was prepared using the same procedure.

Animals and dietary regime

Forty eight male Wistar rats (150-200 g) were used for the study. The animals were placed in the experimental room (Bombay college of Pharmacy, Mumbai, India) under standard conditions of temperature ($25 \pm 2^\circ\text{C}$) and relative humidity ($55 \pm 10\%$). Animals were subjected to 12:12 light/dark cycle. All the experiments were performed after approval of the protocol by the Institutional Animal Ethics Committee (IAEC; Approval No: CPCSEA-BCP/2009-19) and were carried out in accordance with the current guidelines for the care of laboratory animals, CPCSEA laid down by the Government of India.

Intratracheal administration of CSE

All the rats received a single intratracheal dose of CSE (1.3 mL/kg) under ketamine:xylozine anaesthesia (80:20 mg/kg body weight, i.p.) as described previously⁷. Trachea of the animals was

exposed by a minor surgery on ventral side of the neck, under anaesthesia, and the CSE then injected into the trachea by piercing a sterile syringe needle in between two cartilaginous tracheal rings. Control group was treated in the same manner with intratracheal injection of PBS pH 7.4 (1.3 mL/kg).

Protocol and treatment

Forty eight male Wistar rats weighing between 150-200 g were divided into eight groups (n=6) for following experiments. Air bubbled phosphate buffered saline (PBS) and CSE were administered intratracheally (i.t.), while capsaicin as oleoresin and all other study drugs were administered intraperitoneally (i.p.). Capsaicin was given 30 min prior to administration of CSE. Groups V-VIII were treated with study drugs followed by capsaicin further followed by CSE instillation with 30 min time interval between each exposure. Group details are as follows: Group I, phosphate buffered saline (PBS, 1.3 mL/kg) (vehicle control); Group II, CSE (1.3 mL/kg); Group III, capsaicin (20 mg/kg); Group IV, capsaicin (20 mg/kg) + CSE (1.3 mL/kg); Group V, capsazepine (10 mg/kg) + capsaicin (20 mg/kg) + CSE (1.3 mL/kg); Group VI, rimonabant (3 mg/kg) + capsaicin (20 mg/kg) + CSE (1.3 mL/kg); Group VII, (R)-(+)-WIN 55,212-2 (3 mg/kg) + capsaicin (20 mg/kg) + CSE (1.3 mL/kg); and Group VIII, lidocaine hydrochloride (1 mg/kg) + capsaicin (20 mg/kg) + CSE (1.3 mL/kg).

Bronchoalveolar lavage fluid (BALF) collection, cell counting and protein estimation

BALF collection was performed after 24 h of CSE administration as described previously⁷. The BALF was centrifuged at 3000 rpm for 10 min at 4°C. The pelleted BALF cells were re-suspended in PBS, and total leukocyte count was counted using standard hemocytometer. Cell classification was examined by counting 100 cells on a smear prepared by cytospin and Wright-Geimsa staining. Total protein content was determined in BALF supernatant with the help of liquixx total protein kit (ERBA diagnostics Mannheim GmbH). After BALF collection, lung tissue was collected, perfused with ice-cold phosphate buffered saline (PBS) and stored at -70°C until homogenisation.

Measurement of MPO activity and TNF α levels in lung tissue supernatants

Lung homogenates (10%) were prepared by homogenization of lung tissue in 50 mM phosphate buffer pH 6 containing 0.5% Hexadecyl-trimethyl-

ammonium bromide (HTAB). After homogenisation of the lung tissue, the lung tissue supernatants were processed for the estimation of myeloperoxidase (MPO), matrix metalloproteinase 9 (MMP 9) activities and histopathological examination. Myeloperoxidase activity was determined as previously described^{21,22}. TNF α levels were measured with the aid of ELISA kit (Quantikine Immunoassay, R&D Systems).

Measurement of MMP 9 activity in lung tissue supernatants

Lung tissue homogenates (5%) were prepared in ice cold phosphate buffered saline (PBS). MMP 9 activity was determined in the lung tissue supernatants by gelatin zymography as described previously²³. Proteins from each lung supernatant (20 μ g) were separated in 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis containing 1 mg/mL gelatin (Bovine Skin, Sigma Aldrich) using Mini Protean tetra gel system (Bio-Rad laboratories). Gels were washed with 1% Triton X-100, developed in Tris buffer containing 5 mM CaCl₂ and 5 μ M ZnCl₂ and stained with 0.5% w/v Coomassie blue solution. Bands of MMP-9 gelatinolytic activities were analyzed with Image Lab software in Gel Doc XR+ (Bio-Rad laboratories).

Histopathological examination

Tissues specimens were collected from animals belonging to different treatment groups. After collection, the tissues were immediately preserved in the 10% neutral buffered formalin, processed by routine method for histological observation. Processed tissue were sectioned (at 5 μ m) and taken on the clean glass slides and stained by hematoxylin and eosin and observed under microscopes at 100X magnification.

Statistical analysis and expression of results

Results are expressed as mean \pm SEM (n=6). One-way ANOVA with Bonferroni's post test was performed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA. **P* < 0.01 was considered significant

Results and Discussion

Effect of pretreatment of study drugs on deviant effect of dual exposure to capsaicin and CSE on TLC, DLC and protein content of BALF

Rats exposed to capsaicin and CSE showed a significant increase, 7.7 ± 0.03 in the total leukocyte count (TLC) of BALF measured as ($\times 10^6$) cells/mL BALF as compared to the vehicle control group, 1.36 ± 0.01 . Pretreatment with study drugs significantly reduced the TLC of BALF to 5.88 ± 0.01 , 5.6 ± 0.02 ,

5.71±0.01 and 5.51±0.03 with capsazepine, rimonabant, WIN 55,212-2 and lidocaine groups, respectively (Fig. 1A). Measurement of DLC showed a significant rise in the counts of lymphocytes, macrophages and neutrophils (Table 1). Lymphocytes increased from 0.76 ± 0.01 of vehicle control group to 3.51 ± 0.01 of coexposure group of capsaicin and CSE which was significantly higher than the CSE group, 2.64 ± 0.02 or capsaicin group, 2.73 ± 0.01 when both were employed as a single offending agent. Lymphocytes were significantly reduced to 1.07 ± 0.01, 1.04 ± 0.00, 1.14 ± 0.01 and 1.21 ± 0.01 with capsazepine, rimonabant, WIN 55,212-2 and lidocaine groups, respectively. Macrophages in coexposure group increased drastically to 1.87 ± 0.02 from 0.34 ± 0.00 of vehicle control group and also as compared to 1.29 ± 0.04 of CSE group and 1.27 ± 0.00 of capsaicin group.

Macrophages showed significant reduction to 1.07 ± 0.01 with capsazepine, 1.05 ± 0.01 with rimonabant, 1.13 ± 0.01 with WIN 55,212-2 and 1.08 ± 0.00 with lidocaine. Neutrophils showed significant increase from 0.26 of vehicle control group to 2.33 ± 0.01 of coexposure group and also when compared with CSE group, 1.74 and 1.75 ± 0.01 of capsaicin group (Table 1). Neutrophils counts were reduced significantly in the

drug pretreated groups, capsazepine, 1.04; rimonabant, 1.07±0.01; WIN 55,212-2, 1.13; lidocaine, 1.08±0.01 (Table 1). CSE and capsaicin coexposure considerably increased the protein content of BALF, 1.86±0.02 g/dL as compared to capsaicin or CSE alone exposure, 0.98 ± 0.01 g/dL, 1.67 ± 0.06 g/dL, respectively and the vehicle control group, 0.98 ± 0.01 g/dL. Increased protein content was evidently attenuated by study drugs, capsazepine, 0.93 g/dL; rimonabant, 0.96 ± 0.0040 g/dL; WIN 55,212-2, 0.91 g/dL; and lidocaine, 0.92 g/dL (Fig. 1B).

Effect of pretreatment of study drugs on deviant effect of dual exposure to capsaicin and CSE on MPO, MMP 9 activities and TNF α concentrations in lung supernatants

Rise in MPO activities (U/g of lung tissue) of lung supernatants corroborated with the rise in neutrophils counts of the capsaicin and CSE group, 50.04 ± 1.94 as compared to vehicle control group, 2.12 ± 0.01 and CSE group, 18.18 ± 1.52 and capsaicin group, 22.04 ± 0.59. This rise was prevented with study drugs, capsazepine, 7.53 ± 0.098; rimonabant, 6.90 ± 0.58, WIN 55,212-2, 5.67 ± 0.33 and lidocaine, 7.55 ± 0.40 (Fig 1C). MMP 9 activity of lung supernatants evaluated by zymography was found to be 6.62 ± 0.05 in the capsaicin and CSE

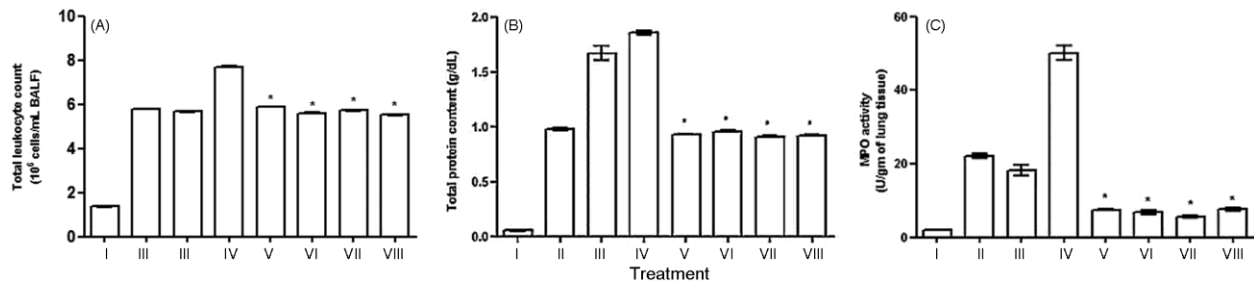


Fig. 1—(A) Total leukocyte count; (B) Total protein content of the bronchoalveolar lavage (BAL); and (C) Myeloperoxidase (MPO) activity of the lung tissue supernatants recovered after 24 h of cigarette smoke extract (CSE) and/or capsaicin exposure. [Results are expressed as (x 10⁶ cells/ml BALF) ± SEM of 6 rats. One-way ANOVA followed by Bonferroni's post test was performed. *P <0.01 was considered significant when the drug pretreated groups were compared with Cap+CSE group]

Table 1—Differential leucocyte counts in Bronchoalveolar lavage fluids (BALF) collected after 24 h in rats exposed to capsaicin (Cap) and cigarette smoke extract (CSE) or Phosphate buffered saline (PBS) and pretreated with various drugs

Groups	Differential cell count (DLC) x 10 ⁶ cells/mL BALF		
	Lymphocytes	Macrophages	Neutrophils
PBS	0.76 ± 0.01	0.34 ± 0.00	0.26 ± 0.00
CSE	2.64 ± 0.02	1.29 ± 0.04	1.74 ± 0.00
Cap	2.73 ± 0.01	1.27 ± 0.00	1.75 ± 0.01
Cap+CSE	3.51 ± 0.01*	1.87 ± 0.02*	2.33 ± 0.01*
Capsazepine+Cap+CSE	1.07 ± 0.01 [#]	1.07 ± 0.01 [#]	1.04 ± 0.00 [#]
Rimonabant+Cap+CSE	1.04 ± 0.00 [#]	1.05 ± 0.01 [#]	1.07 ± 0.01 [#]
WIN 55,212-2+Cap+CSE	1.14 ± 0.01 [#]	1.13 ± 0.01 [#]	1.17 ± 0.00 [#]
Lidocaine+Cap+CSE	1.21 ± 0.01 [#]	1.08 ± 0.00 [#]	1.16 ± 0.01 [#]

[Data is expressed as mean ± SEM of 6 rats. One-way ANOVA followed by Bonferroni's post test was performed and *P < 0.01 was considered significant when capsaicin and CSE (Cap+CSE) group compared with control PBS group. [#]P <0.01 was considered significant when the drug pretreated groups were compared with Cap+CSE group]

group which was significantly higher than in the vehicle control group, 1.01 ± 0.01 and also CSE group, 4.53 ± 0.28 and capsaicin group, 3.49 ± 0.09 per se. This was considerably reduced with capsazepine, 1.70 ± 0.01 ; rimonabant, 1.91 ± 0.01 ; WIN 55,212-2, 1.79 ± 0.03 and lidocaine, 1.14 ± 0.03 (Fig. 2A, B). Intratracheal coadministration of capsaicin and CSE caused a considerable increase in the lung TNF α levels, 40.83 ± 0.75 pg/mL after 24 h as compared to the vehicle control group, 3.5 ± 0.43 pg/mL and also single induction by CSE, 30 ± 0.77 pg/mL or by capsaicin, 34.67 ± 0.88 pg/mL. TNF α levels were markedly reduced to 5.33 ± 0.21 , 10.83 ± 0.31 , 16.17 ± 0.31 and 5.83 ± 0.31 with capsazepine, rimonabant, WIN 55,212-2 and lidocaine treatments, respectively (Fig. 3).

Effect of pretreatment of study drugs on deviant effect of dual exposure to capsaicin and CSE on the histopathology of lung tissue.

Histopathological evaluation revealed disrupted and inflamed features in lung tissues on the dual exposure, capsaicin and CSE group (Fig. 4) as compared to the vehicle control group. The lung histology was evidently found to be improved in study drugs pre-treated animals in terms of lesser presence of perivascular infiltration (PVI) of the inflammatory cells.

The current study investigated the effect of capsazepine, rimonabant, WIN 55,212-2 and lidocaine

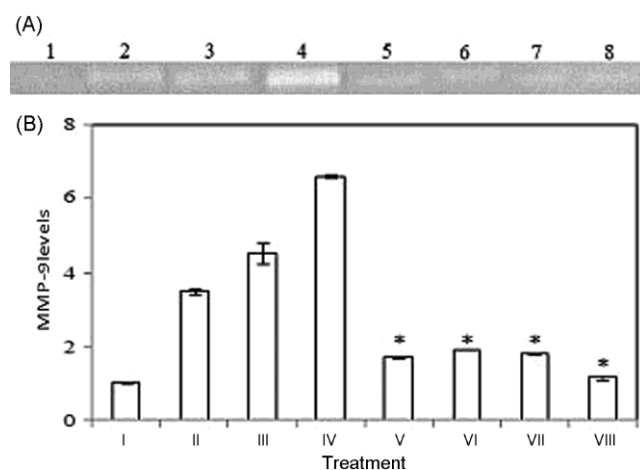


Fig. 2—(A) Representative gelatin zymograms performed with samples of lung tissue supernatants after 24 h exposure to the group I-VIII (Details in M & M). Air bubbled PBS and CSE were administered intratracheally, while capsaicin as oleoresin and all other study drugs were administered intraperitoneally; and (B) MMP 9 activities of the lung tissue supernatants recovered from rats after 24 h of cigarette smoke extract (CSE) and/or capsaicin exposure. [Results are expressed as U/g of lung tissue \pm SEM of 6 rats. One-way ANOVA followed by Bonferroni's post test was performed. * $P < 0.01$ was considered significant when the drug pretreated groups were compared with Cap+CSE group]

on acute lung inflammation induced with co-exposure of capsaicin (i.p.) and CSE (i.t.).

TRPV1 receptor functions as a sensor for detecting irritants in the lung by transmitting noxious stimuli to the central nervous system and inducing the release of a variety of proinflammatory neuropeptides at the peripheral terminals thus also acting as integrator of inflammatory signalling²⁴. TRPV1 receptor activation leads to an influx of Ca^{2+} and prolonged application of an agonist, such as capsaicin, found in spicy food leads to release of central transmitters (glutamate and substance P) from nociceptive afferents resulting in desensitization of the channel. As a result of exhaustion of transmitters and desensitization, the afferent becomes 'chemically denervated' and functionally silent²⁵. Capsaicin, a TRPV1 agonist is known to be an important mediator of inflammation. Also, CSE-induced murine model mimicked a COPD-like lung injury⁹ CS associated lung injury is characterized by inflammation and vascular remodelling. In addition, CS is a strong environmental risk factor linked to other inflammatory conditions too²⁶. Hence, exposure to both the lung irritants, one a food component and other an inhalant was found to be prudent to develop an acute lung inflammatory model mimicking COPD. It was employed to evaluate the effects of drugs based on the hypothesis that the drugs are acting via a common mechanism of TRPV1 gating.

Coexposure to intraperitoneal capsaicin and intratracheal CSE lead to development of acute lung inflammation within 24 h in Wistar rats indicated that there was pronounced increase in the BALF cell counts

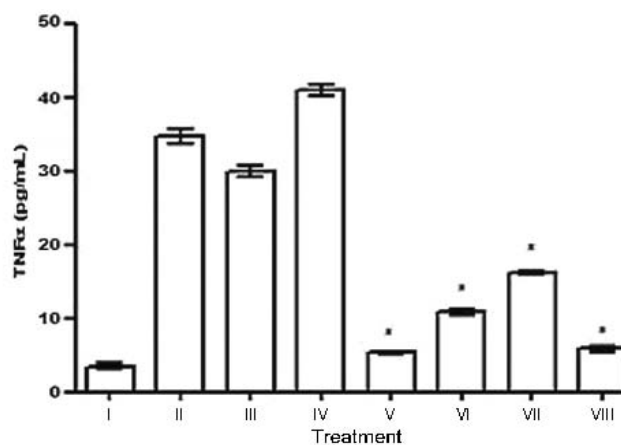


Fig. 3—TNF- α concentration of the lung tissue supernatants recovered from rats after 24 h of cigarette smoke extract (CSE) and/or capsaicin exposure. [Results are expressed as pg/mL \pm SEM of 6 rats. One-way ANOVA followed by Bonferroni's post test was performed. * $P < 0.01$ was considered significant when the drug pretreated groups were compared with Cap+CSE group]

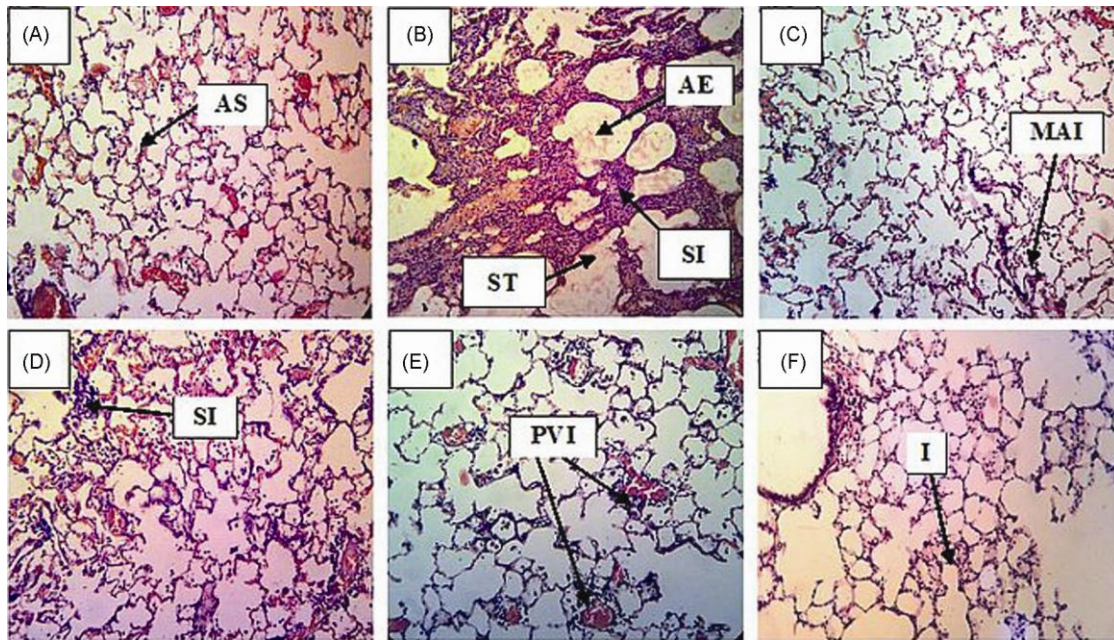


Fig. 4—Histological images showing lung sections stained with hematoxylin and eosin. (A) represents control; (B) capsaicin+CSE; (C-F) Groups pretreated with lidocaine (1 mg/kg), rimonabant (3 mg/kg), WIN 55,212-2 (3 mg/kg) and capsazepine (10 mg/kg), respectively. [(A) Shows no abnormality (AS, alveolar septum; A, alveolus); (B) Shows severe infiltration of inflammatory cells in interalveolar septum (SI), increased septal thickening, alveolar and septal edema (AE); (C & D) Shows mild infiltration of inflammatory cells in alveolar lumen (MAI) and interalveolar septum (SI); (E) Shows mild perivascular infiltration (PVI) of the inflammatory cells; and (F) Shows moderate focal infiltration (I) of inflammatory cells in alveolar lumen and interalveolar septum, focal increase in septal thickening (Original magnification x 100)]

in the capsaicin and CSE group as compared to capsaicin or CSE groups per se exhibiting WBC migration into lung spaces which is proven to be involved in genesis and progression of lung diseases especially COPD²⁷. Increased DLC measurement is specifically linked to lung damage. Venet *et al.*²⁸ showed increased lymphocytes are specifically recruited to the lung during acute lung injury, and that there is a specific and non-redundant role for each lymphocyte subpopulation in the pathophysiology to the extent of being in control of neutrophils recruitment to the site of inflammation. Macrophages also have a known important contribution towards lung injury via their ability to produce nitric oxide²⁹. Neutrophils are among the earliest leukocytes to traffic into inflammatory sites and are potent amplifiers of early inflammation³⁰. The study drugs have effectively reduced the DLC in BALF proving themselves efficacious in abating acute lung injury. There was marked elevation in the total protein content of BALF in the capsaicin and CSE group as compared to capsaicin or CSE exposure indicating plasma protein extravasation³¹. TNF- α levels, MPO and MMP 9 activities too were elevated in the capsaicin and CSE group. The histopathological changes revealed that there was severe lung inflammation in the capsaicin plus

CSE group as opposed to moderate inflammation in the capsaicin or CSE alone exposure groups. All the four drugs under study, capsazepine, rimonabant, WIN 55,212-2 and lidocaine belonging to diverse pharmacological classes have been successful in relieving the lung inflammation caused by capsaicin and CSE alone and also by the dual exposure.

It is now well established that a wide variety of pro-inflammatory mediators and growth factors released/recruited at the site of tissue injury and inflammation exert extensive post-translational modifications in the TRPV1 channel protein, mainly by phosphorylation³². These mediators activate their respective receptors expressed on sensory nerve fibres and lead to sensitization of TRPV1 channel activity and expression, mainly via the activation of protein kinases A and C (PKA, PKC), p38 mitogen activated protein kinase (MAPK) and Src³³⁻³⁸. Several cytokines and chemokines, eg. TNF α has been shown to modulate TRPV1 function and decrease the thermal activation threshold of the channel^{39,40}. Recently, it was shown that TNF α stimulates MMP-9 gene expression which is mediated through NF- κ B upregulating MMP-9 promoter activity, involving TNFR1, c-Src-dependent MAPKs [i.e. Extracellular

signal regulated kinase (ERK 1/2), p38 MAPK, and Janus kinase (JNK 1/2)] and c-Src-independent IKK (IKB Kinase)/NF- κ B pathways. All the above help construe the mechanism behind tissue remodelling occurring in lung inflammatory conditions such as COPD, asthma, emphysema, etc. where proinflammatory markers like TNF α stimulates MMP-9 which is mediated through NF- κ B and all converging at TRPV1 channel protein. It correlates well with our experimental results where both TNF α levels and MMP-9 activities increased with dual exposure of capsaicin and CSE as compared to solo exposures and was found significantly reduced after pretreatment with study drugs. Since, TRPV1 emerges to be the relevant hub of inflammation, blocking it appears to be a rational anti-inflammatory approach which is delivered by capsazepine. While, rimonabant is a cannabinoid receptor, CB1 antagonist; cannabinoids activate numerous signalling pathways because of their binding to both G protein-coupled receptors (GPCRs), such as CB1 and CB2, as well as ionotropic receptors, such as TRPV1, TRPV4, and TRPA1⁴¹. Blockade of CB1 receptor by rimonabant might probably result in the release of anandamide which in turn may activate and quickly desensitize TRPV1.^{14, 15} Recently, it was reported the prototypical cannabinoid agonist WIN 55,212-2 reduced the secretion of matrix metalloproteinase-9 (MMP-9) in a murine model of cigarette-smoke induced lung inflammation reinstating that cannabinoids are involved in the regulation of inflammatory processes¹⁶. The study also revealed that TRPV1 was involved in inhibition of secretion and intracellular accumulation of MMP-9 upon WIN-treatment. Further, local anaesthetic (LA), lidocaine was evaluated based on the study of Leffler *et al.*¹⁷ that showed LAs activated and sensitized TRPV1, and thus add one more to the growing list of compounds that gate TRPV1. Further elucidation of action of LAs on TRPV1 gating was shown by Rivera-Acevedo *et al.*¹⁸ revealing that TRPV1 is a novel target for LAs to reach their intracellular binding site on the voltage-gated Na⁺ channel. They have shown LAs exhibit a dichotomous action by inhibiting activation of TRPV1 channels in the presence of capsaicin whereas at higher concentrations in the millimolar range appear more relevant to clinical regional anesthesia and neural blockade¹⁸.

Thus, the investigational drugs have significantly alleviated inflammation induced by capsaicin and/or CSE exposures. Although the drugs have different upstream mechanisms, they possess a common

property of direct or indirect action on TRPV1 channels which may prove their potential to be useful in targeting lung inflammatory diseases like asthma and COPD. Thus, our study empirically confirms the use of capsazepine, rimonabant, WIN 55,212-2 and lidocaine in reducing acute lung inflammation. Clinical studies are needed to substantiate the use of these drugs in inflammatory lung disorders.

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