

# Inhalable Solubilized Zileuton for Improved Lung Targeting- *in vitro* and *in vivo* Analysis

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## ABSTRACT

**Background:** Pulmonary diseases that affect the normal functioning of the lungs show airway symptoms ranging from change in airflow to bronchiectasis. Zileuton is an inhibitor of 5-lipoxygenase enzyme that catalyzes the synthesis of leukotrienes. Zileuton is used in the management of inflammatory conditions of the upper airways like obstructive pulmonary conditions and acute lung inflammation. Although a promising therapeutic, zileuton is poorly water-soluble and requires frequent administration to overcome bioavailability issues and maintain therapeutic levels that often lead to adverse reactions, especially to the non-targeted organs. **Materials and Methods:** Therefore, we designed a rapidly nanoemulsifying formulation of zileuton using Acrysol K150 as an oil, Cremophor EL as a surfactant, and Transcutol HP as a cosolvent. **Results:** This self-emulsifying composition exhibited showed a mean globule size of  $133 \pm 3.6$  nm with a polydispersity index of 0.38. Scanning electron microscopy (SEM) images revealed the spherical shape of emulsion globules. *In vitro* lung deposition showed >80% delivery to the deep lung tissue. Mass median aerodynamic diameter of  $2.05 \pm 0.98$   $\mu$ m for the aerosolized formulation. *In vivo*, pharmacokinetic studies in Wistar rats by inhalation route showed that the zileuton-loaded nanoemulsifying formulation had a significantly higher concentration in the lung compared to other non-target organs. The *in vivo* efficacy in the lipopolysaccharide-induced acute lung inflammation model in rats significantly impeded the protein accumulation and neutrophil infiltration in the lungs. **Conclusion:** The zileuton-loaded nanoemulsifying inhalable formulation successfully improved the therapeutic efficacy of zileuton specifically to the lung thereby minimizing the off-target organ side effects.

**Keywords:** Self-emulsifying, Zileuton, Aerosols, Pharmacodynamics, *in vivo*, Lung targeting.

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## INTRODUCTION

In developing countries, chronic diseases of the lungs are among the leading causes of death. An infection of the respiratory system commonly causes morbidity, which deteriorates the health conditions already present.<sup>1</sup> The effects of asthma on the lungs and the narrowing of the airways are wheezing, chest tightness, and shortness of breath. Patients with the aforementioned poor health conditions and other forms of injury like acute respiratory distress syndrome/ acute lung injury (ARDS/ALI) require frequent hospitalization, which contributes to major global health burdens and greatly affects the national economy involving all age groups.<sup>2</sup>

Inflammatory mediators like leukotrienes induce numerous biological effects, including neutrophil and monocyte aggregation, eosinophil migration, and augmentation of neutrophils. Change in capillary permeability and contractions of smooth muscles are also observed.<sup>3</sup> These inflammatory effects eventually increase mucus secretion, edema, and bronchoconstriction.<sup>4</sup> Leukotrienes play major role as the mediators of inflammation and allergy. In chronic cases, they are also involved in the pathogenesis of cancer and cardiovascular diseases.<sup>5,6</sup> Inhibition of leukotriene synthesis is one of the best way to manage inflammatory conditions that are leukotriene-mediated.

Zileuton inhibits enzyme 5-lipoxygenase. Lipoxygenase enzyme catalyzes the formation of leukotrienes from arachidonic acid and thus hinders leukotriene synthesis.<sup>7</sup> It also plays a therapeutic role in patients with aspirin-induced asthma, COPD and lung inflammation.<sup>8,9</sup> Zileuton is also used in the management of



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pulmonary dysfunction, airway inflammation and airway smooth muscle remodeling.<sup>10</sup> Zileuton has a low water solubility, thus liquid formulations requiring drug concentrations above its aqueous solubility limit may need to add pharmaceutically approved organic cosolvents. Several attempts have been made to increase the solubility of zileuton.<sup>11,12</sup> Self-emulsifying systems contain lipid, that helps to solubilize lipophilic drug. These formulations are easy to manufacture and scale-up as they need only low shear mixing equipments. As they contain lipid and surfactant, poor bioavailability and permeability issues of many drugs can be easily handled. Nanoemulsifying systems can specifically target drugs to the inflamed organs because of their lipophilicity, and smaller particle size that can drain via enhanced permeability and retention effect. These systems also accommodate high amount of lipophilic drug, and also offer good physical stability.<sup>13</sup> The surfactant benefits the formulation by increasing membrane permeability, while oils enhance lymphatic transport leading to greater targeting efficiency.<sup>14,15</sup> Use of surfactants also facilitate pulmonary drug delivery as they act as absorption enhancers.<sup>16</sup> We examined the solubility and stability of zileuton. For that we constructed a ternary solvent system. Three ingredients namely oil, surfactant, and cosolvent were screened in various concentration ratios. Physicochemical characterizations confirmed the small size, uniform distribution and desirable aerodynamic properties. The biodistribution of zileuton on different organs (i.e., lung, liver and spleen) of Wistar rats have also been investigated before proceeding with *in vivo* studies in diseased animal model. This study suggested the capability of the prepared zileuton-loaded self-nanoemulsifying system to lower the inflammation markers and inhibition of neutrophils in bronchoalveolar lavage fluid, suggesting usefulness in lung delivery.

## MATERIALS AND METHODS

### Materials

Acrysol K-150 and Cremophor EL were obtained from Coral Pharma Chem. (Ahmedabad, India) and BASF, India. Zileuton was a gift sample by Emcure Pharmaceuticals, Pune, India. Cosolvents such as polyethylene glycol (PEG), propylene glycol (PG), and Transcutol HP were obtained from Merck Chemicals Mumbai and Gattefosse, Mumbai, India. Methanol, Tween 20, Tween 80, normal saline and phosphate buffer saline were purchased from Himedia, Mumbai, India. Lipopolysaccharide (LPS) from *Escherichia coli* O111:B4 was purchased from Sigma-Aldrich (St. Louis, MO, USA). Biochemistry analysis kits were purchased from Sigma Aldrich and Cayman Chemical Company.

## Methods

### Formulation of Zileuton SEDDS and Estimation of drug loading

Equilibrium solubility measurements of zileuton were carried out in various excipients using shake flask method. Zileuton showed the highest solubility in Acrysol K-150, Cremophor EL and Transcutol HP among oils, surfactants and cosolvents, respectively. Different ratios of oils, surfactants and cosolvents were screened for requisite preconcentrates formulation.<sup>17,18</sup> A series of self-emulsifying preconcentrates including Acrysol K-150 as the oil phase, Cremophor EL as the surfactant, and Transcutol HP as the cosolvent were developed based on the solubility data. Further, Zileuton (100 mg/mL) was added to optimized preconcentrate and thoroughly mixed for 30 min. The influence of aqueous dilution on zileuton-loaded preconcentrate was analyzed through titration with distilled water. The mixtures after dilution with water were monitored visually homogeneity and phase separation if any. The formulation was evaluated for cloud point, refractive index, and transmittance as per the procedures reported earlier.<sup>18</sup>

### Estimation of zileuton

The validated RP-HPLC method was used to estimate the zileuton content.<sup>19</sup> The chromatographic system was operated in an isocratic elution mode. Mobile phase was used to dilute test samples. Methanol: Ortho phosphoric acid (0.1%) (80:20) was the composition of mobile phase. Flow rate was of 1 mL/min. The separation was carried out at ambient temperature on a reversed-phase C<sub>18</sub> column. Injection volume was of 20 µL. Detector was set at 260 nm. According to the recommendations, the procedure was validated for a wide range of variables.

### Scanning electron microscopy and particle size distribution studies

The formulation was diluted with double distilled water before examination in order to measure mean globule size and surface charge. By using photon cross-correlation spectroscopy (Nanophox, Sympatec, Germany) at a scattering angle of 90°, the mean globules were determined.<sup>20,21</sup> Zeta analyzer (Delsa Nano C, Beckman Coulter, Japan) surface charge potential was measured. Scanning electron microscopy (SEM) imaging was performed at different magnifications.

### Twin Stage Impinger (TSI) and Anderson Cascade Impactor-based *in vitro* lung deposition (ACI)

The post nebulization droplet size distributions were estimated through glass twin stage impinger (TSI). The Anderson Cascade Impactor was used to estimate the geometric standard deviation (GSD), emitted dose (ED), mass median aerodynamic diameter (MMAD) and fine particle fraction (FPF) (ACI). Nano-emulsifying zileuton formulation was diluted with

phosphate buffer saline (PBS) and used for analysis. Tests were run in accordance with the method described in the literature. Lab micropump nebulizer (Aeroneb®) was used to nebulize the reconstituted sample.<sup>22</sup>

### Targeting efficacy, biodistribution, and pharmacokinetics

Wistar rats ( $n = 24$ ), of either sex, weighing  $220 \pm 20$  g were housed in a temperature-controlled environment at  $25 \pm 2^\circ\text{C}$  with a 12-hr natural light/dark cycle. Eight-week-old, healthy laboratory-bred rats were given an unlimited supply of tap water and a commercial pellet diet. The experiments were carried out in an animal house that had been approved by India's Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA). Two groups were randomly assigned to deliver conventional zileuton formulation and zileuton-loaded nano emulsifying systems. The animals were given free access to water the night before the experiment while fasting. The formulations (10 mg/kg body weight of zileuton) were then nebulized using a small animal nebulizer. The formulations in both groups were zileuton-loaded nano emulsifying systems and zileuton oral dispersion. Rats were sacrificed to obtain blood and organ samples at each predetermined time point. Immediately, plasma and vital organs, such as the spleen, liver, lungs and were extracted from the animals in order to calculate the zileuton concentration and thus the targeting efficiency. The harvested organs were processed to extract the zileuton.

The following parameters were calculated: relative uptake rate ( $r_e$ ), drug targeting efficiency ( $t_e$ ), and relative selectivity ( $r_{te}$ ):<sup>23,24</sup>

$$r_e = \frac{(\text{AUC } \infty) \text{ test targeted delivery system}}{(\text{AUC } \infty) \text{ conventional delivery system}} \text{ -----1}$$

$$t_e = \frac{(\text{AUC } \infty) \text{ target tissue}}{(\text{AUC } \infty) \text{ non target tissue}} \text{ -----2}$$

$$r_{te} = \frac{(t_e) \text{ test target drug delivery system}}{(r_e) \text{ conventional drug delivery system}} \text{ -----3}$$

### LPS-induced lung inflammation and rat *in vivo* efficacy testing

Animal studies received approval from the Institutional Animal Ethics Committee (IAEC). There were four groups formed, each with six rats: standard treatment, and test treatment normal control and diseased control.<sup>23</sup> Normal saline was used to dissolve LPS. After sterile filtration, it was administered intratracheally at a dose of 5 mg/kg body weight.<sup>25</sup> Normal control group received normal saline (0.9% w/v sodium chloride in water) intratracheally and not the LPS solution. No treatment was given to normal control and diseased control group. Normal and diseased control groups received no treatment. The standard treatment and test treatment groups inhaled zileuton dispersion in saline and zileuton loaded nano-emulsifying system reconstituted in normal saline, respectively (Equivalent to zileuton 1 mg/kg body weight).

### Evaluation of inflammatory biomarkers in bronchoalveolar lavage fluid (BAL)

Analysis of total protein, neutrophils (%), and pulmonary hemorrhage were performed on bronchoalveolar lavage fluid (BALF). TNF-, IL-1, IL-6, and TBARS, as well as other chemicals, were also examined in the BALF.<sup>21,22</sup>

### Statistical analysis

Each experiment was carried out three times, and the mean and standard deviation of the outcomes were presented. The statistical significance of the data was assessed using one-way analysis of variance (ANOVA) with a 95% level of confidence. The Newman-test Keul's for statistical significance at a 95% confidence level was used to assess any significant differences between groups.

## RESULTS AND DISCUSSION

### Physicochemical properties of nano self-emulsifying delivery system of zileuton

To deliver a drug successfully through self-emulsifying system, it should get adequately solubilized in the excipient used in the formulation. If the drug solubility is insufficient, there is a risk of drug precipitation during aqueous dilution and administration to the body. Therefore, a critical consideration for self-emulsifying formulations is the drug's solubility in the excipients. We performed zileuton equilibrium solubility tests in each lipid excipient. Among the numerous oils, surfactants, and cosolvents studied, zileuton demonstrated the maximum solubility in Acrysol K-150, Cremophor EL, and Transcutol HP, respectively. Among the various combinations of oil, surfactant and co-surfactants tried, a system containing 90:5:5 of Acrysol K-150:Cremophor EL:Transcutol HP was selected for further analysis. The nanoemulsifying system was diluted with double distilled water to generate a nanoemulsion. Initially a dummy

**Table 1: Evaluation parameter of nano self-emulsifying systems with and without zileuton loading (Mean of six determinations).**

Evaluation parameters	Dummy formulation	Formulation with zileuton
Mean globule size (nm)	128.12	133.07
Polydispersity index	0.375	0.382
Refractive index	1.333	1.332
Transmittance (%)	97.56	96.34
Cloud point ( $^\circ\text{C}$ )	90	89

The polydispersity index was calculated to know about the globule size distribution. Zileuton-loaded nanoemulsion showed globule size of  $133 \pm 0.28$  nm ( $n=6$ ) (Figure 1A).

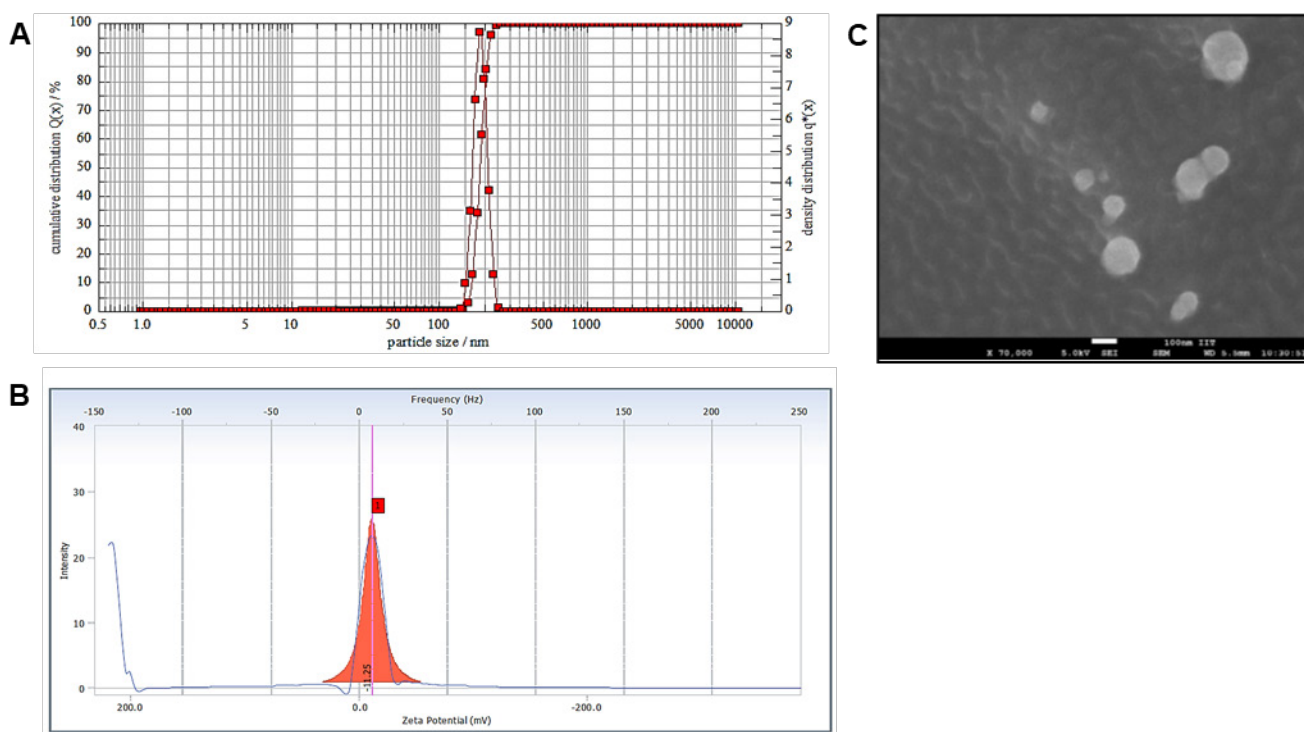
self-nanoemulsifying composition was prepared and evaluated for physicochemical properties. Thereafter, zileuton was added to it and the effect of addition of drug on nanoemulsion characteristics was analyzed (Table 1).

Lower magnitude of polydispersity index of 0.382 indicates narrow size distribution. The surface charge ( $\zeta$ ) of the nanoemulsion plays a role in its stability.<sup>26,27</sup> In general, the surface charge of the droplet is negative due to the presence of acidic groups from fatty acids in the structure of the excipients used.<sup>26</sup> The formulation had 3 components namely, Acrysol K-150 as the oil and Cremophor EL as the surfactant with co-solvent Transcutol HP. The emulsion formed after reconstitution with water was negatively charged with zeta potential  $-11.25$  mV (Figure 1B). The anionic groups of the fatty acids and glycols found in the oil, surfactant, and co-surfactant may also be the cause of the negative charge on the emulsion globules. Higher zeta potential magnitudes are desirable for stability purpose. The more the charge on particles, the lesser will be the chances of flocculation or coagulation. Thus

the system will remain stable on storage as well as *in vivo* in the biological environment. Coalescence and Ostwald ripening reduce nanoemulsion stability.<sup>28,29</sup> SEM image (Figure 1C) showed spherical particles with uniform size distribution and absence of any particle aggregation. Difference in the potential leads to Ostwald ripening. Small particles fuse with larger ones resulting in growth of particles.<sup>30</sup> Cremophor has the better emulsifying capacity and enhances stability. Furthermore, several studies have found that Cremophor EL in self-nanoemulsifying drug delivery systems improves drug permeability and bioavailability.<sup>31</sup>

### In vitro lung deposition testing

The TSI simulates the inhalation process. The pulmonary aerosol particles are passed through a system in which airflow changes its directions like it travels through nose *in vivo*. Based on the size of particles, particles will travel through the obstacles. If particles are large enough they will colloid with the liquid surfaces, similar to oropharynx deposition *in vivo*. Consequently, it demonstrates usefulness in the quick screening of aerosol deposition patterns.<sup>32</sup>



**Figure 1:** A representative particle size distribution and zeta potential graph obtained for zileuton loaded self-nanoemulsifying composition.

**Table 2: Comparative biodistribution of zileuton in the lung, liver, spleen, and plasma following nebulization of the formulation for zileuton nano-emulsification and the standard zileuton dispersion.**

Body fluid / tissue	$r_e$	Zileuton dispersion (Conventional) $t_e$	Zileuton nano-emulsifying system (Test) $t_e$	$r_{te}$
Plasma	0.95	--	--	--
Lung	15.01	0.65	4.18	17.51
Liver	0.87	0.83	0.21	1.75
Spleen	1.98	0.51	0.41	0.54

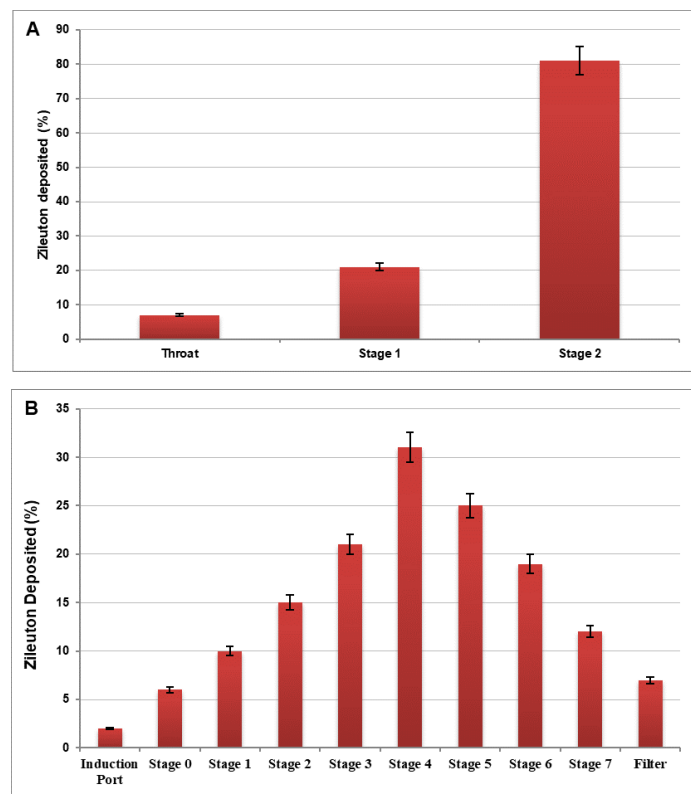
Reduced airway delivery was shown by stage 2 of the twin stage impinger's  $81 \pm 1.0\%$  deposition of the formulation (Figure 2A).

ACI was used to measure the nanoemulsifying formulation's MMAD and GSD when zileuton was added (Figure 2B). Values of  $2.05 \pm 0.98 \mu\text{m}$  and  $1.35 \pm 0.25 \mu\text{m}$ , respectively, were observed. The product that was nebulized had an ED and FPF of  $95.01 \pm 0.59\%$  and  $82.11 \pm 1.01\%$ , respectively. These findings imply that the best aerosol particle size can effectively deposit in the lungs. The developed formulation's aerosol droplets also have a tighter size distribution. According to the findings, zileuton-loaded nanoemulsion has good aerosol characteristics. The developed formulation works well for delivering drugs to deep lung tissue.

### In vivo pharmacokinetic, biodistribution and targeting efficiency

Various parameters derived from the biodistribution profile of the zileuton-loaded nano self-emulsifying system and conventional zileuton have been presented in Table 2 ( $n=6$ ).

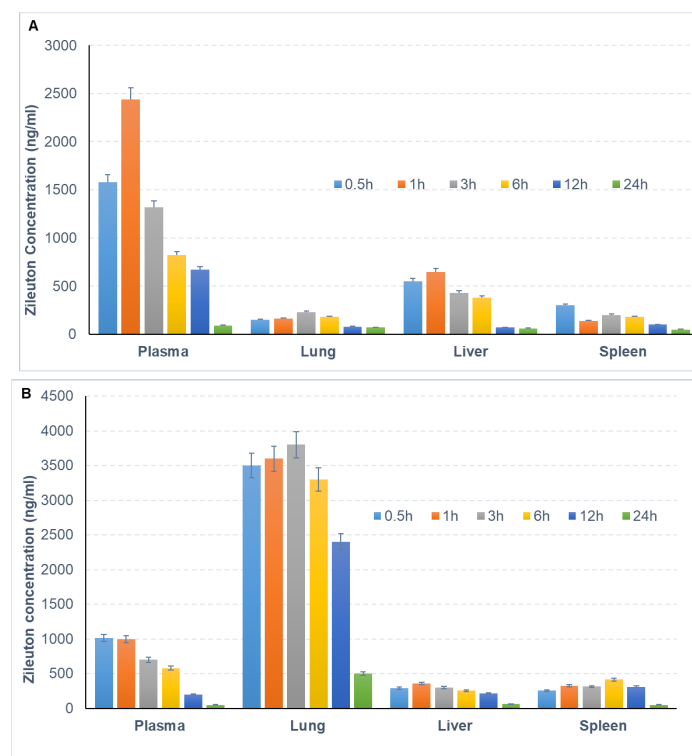
The amount of zileuton in the liver, spleen, lung, and plasma was measured at predetermined intervals over the course of 24 hr. Pharmacokinetic profiles indicated a higher accumulation of zileuton in lungs than liver or spleen for zileuton loaded nanoemulsion (Figure 3).



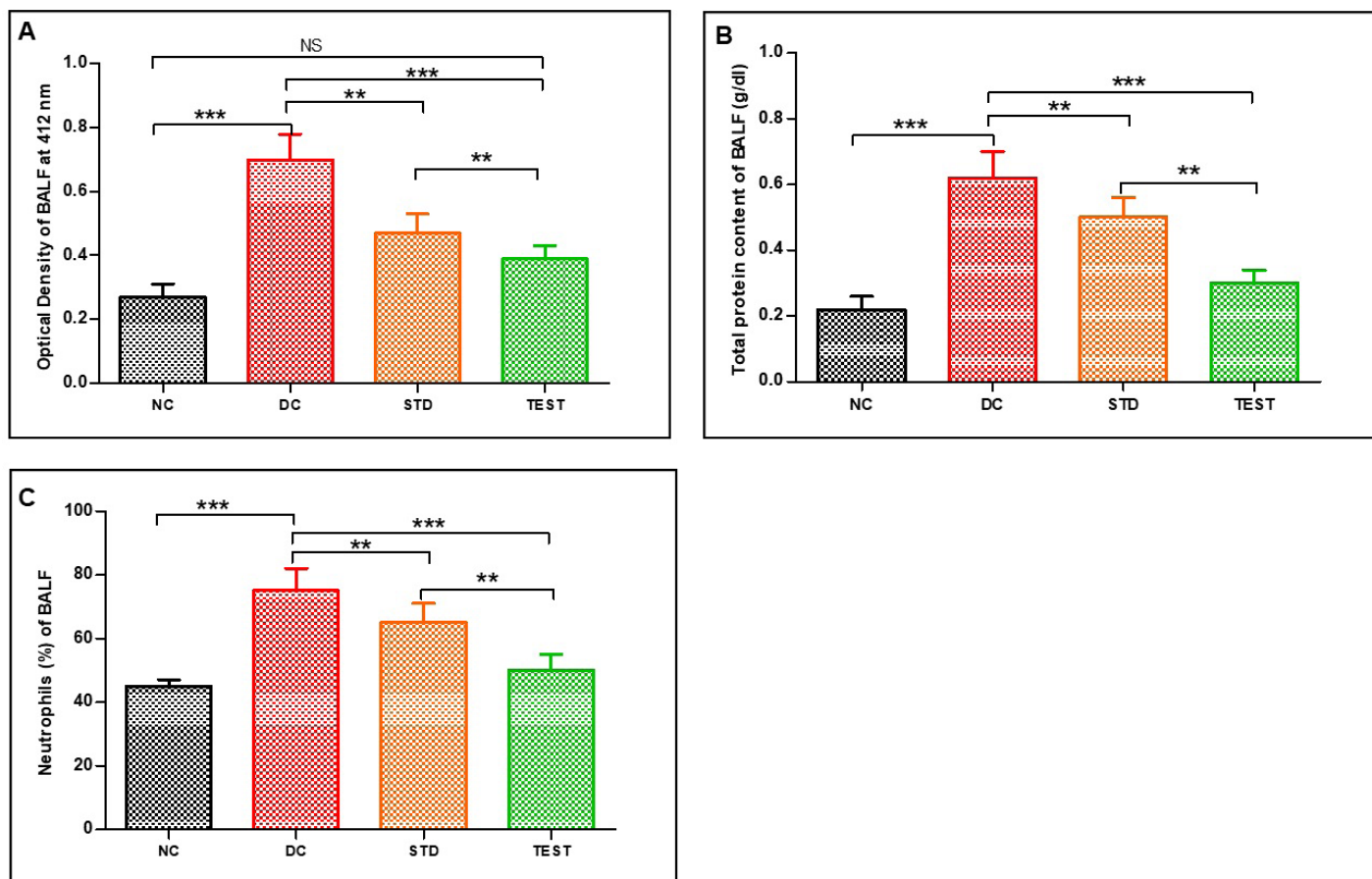
**Figure 2:** A. Deposition in different parts of the lung *in vitro* using TSI. B. At a flow rate of 15 L/min, ACI deposition pattern of zileuton-loaded nanoemulsion on various stages of impactor. ( $n=3$ , error bars indicate standard deviation).

Additionally,  $r_e$ ,  $t_e$ , and  $rt_e$  were calculated to determine the targeting effectiveness of both conventional zileuton and zileuton-loaded nanoemulsion. Higher selectivity for that specific tissue or organ is indicated by a  $r_e > 1$  for that tissue or organ.<sup>33</sup> When zileuton-loaded nanoemulsion was inhaled, the  $r_e$  for the lungs was 15.01. On the other hand, for the liver (1.98 and 0.87, respectively) and plasma (0.95). The current investigation compared the  $t_e$  values for each organ to plasma levels. In contrast to conventional zileuton injection ( $t_e = 0.65$ ), zileuton-loaded nanoemulsion had a  $t_e$  of 4.18. This showed that the zileuton-loaded nanoemulsion distributed better in lung tissue compared to the conventional injection formulation. When the formulation is administered via inhalation, the lower value of  $t_e$  for the liver and spleen shows lower selectivity of nanoemulsifying systems towards them. As a result, the formulation will be slowly removed from the systemic circulation and the medication concentration in the lungs will be optimized for a longer period of time.

The comparative drug targeting capability of two delivery systems was expressed by another parameter,  $rt_e$ , which was determined (herein, zileuton loaded nano-emulsifying systems vs. conventional zileuton oral formulation).<sup>34</sup> The  $rt_e$  values for lung, spleen, and liver were 17.51, 0.54, and 1.75, respectively. This further demonstrated that the zileuton-loaded nanoemulsion's preferred target was the lungs. Furthermore, the zileuton-loaded



**Figure 3:** Zileuton biodistribution in plasma (ng/mL) and tissues like the liver, lungs, and spleen (ng/g) of rats over the course of 24 intervals following oral administration of the standard zileuton formulation (A), as well as Zileuton-loaded lipo-polymeric microspheres. (B) Error bars show the standard deviation, while the numbers represent the mean of three determinations.



**Figure 4:** BALF optical density (A), total protein (B), and neutrophil (C) in BALF from various groups, namely, NC: Normal, DC: Diseased, STD: Standard treatment with zileuton oral. Data is mean of six determinations and error bars indicate standard deviation. \*, \*\* and \*\*\* represent significant differences at  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ , respectively. In comparison to the normal control ( $45 \pm 3\%$ ), BALF from the diseased group (DC) had a significantly higher percentage of neutrophil accumulation ( $81 \pm 5\%$ ). BALF from the standard treatment group contained  $62.5 \pm 5\%$  and  $55 \pm 4\%$  neutrophils, respectively. Notable reduction in neutrophil accumulation for the zileuton-loaded nano-emulsifying formulation treated group (ANOVA,  $p < 0.001$ ) has been observed. The reduced optical density of BALF at a dose of 1 mg/kg indicates substantially decreased hemorrhage and total leucocyte population.

nanoemulsion's preference for the lungs and its lower distribution to other organs indicate less adverse effects and, therefore, safe delivery.

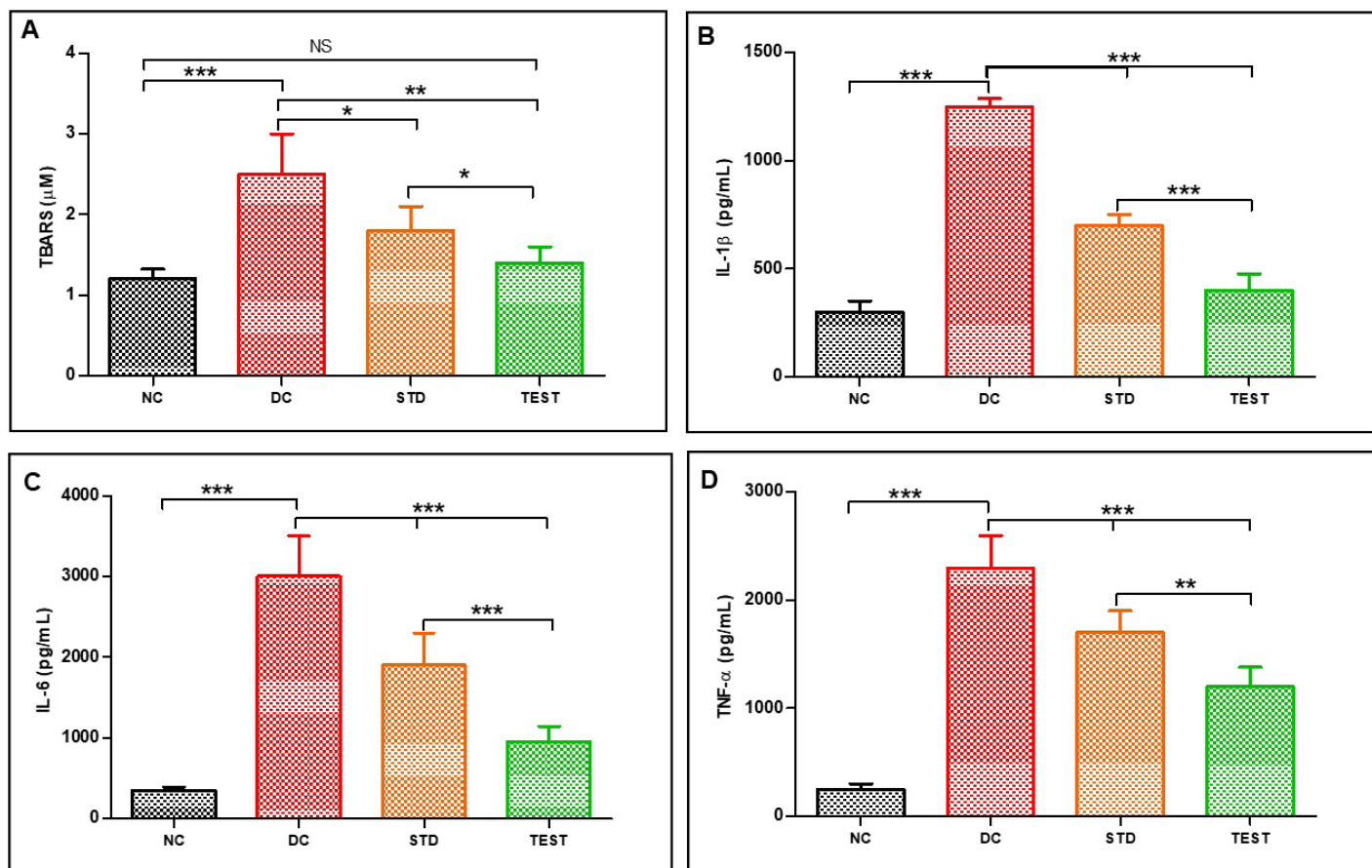
#### ***In vivo efficacy in a model of acute inflammation induced by LPS***

Intratracheally induced acute lung injury model developed using LPS *Escherichia coli* O111:B4. LPS evokes acute inflammation in the lungs. This inflammation over a period of time progresses into severe lung injury. It is the best model to study the efficacy of drug in ALI or ARDS.<sup>22,35</sup> Infected rats were used to assess the therapeutic effectiveness of the zileuton-loaded nanoemulsion and oral dispersion. To verify the therapeutic efficacy of the new formulation, the variation in optical density (indicia of hemorrhagic injury), total proteins, and neutrophils in BALF were measured (Figure 4).

Levels of various inflammatory markers were measured and the data is shown in Figure 5.

TBARS were used to measure lipid peroxidation. BALF from the normal control group contains  $1.20 \pm 0.12 \mu\text{M}$  TBARS ( $p < 0.001$ ). (Figure 5A). The diseased control group had a higher TBARS of  $2.5 \pm 0.5 \mu\text{M}$ , indicating high capillary permeability with neutrophil activation in the alveoli, which increased oxidative stress and caused lung surfactant dysfunction. Lower TBARS values in BALF for zileuton-treated groups indicate that it has an anti-inflammatory and anti-oxidative stress effect.

However, a more prominent effect was observed for the groups treated with conventional zileuton formulation ( $1.80 \pm 0.2 \mu\text{M}$ ,  $p < 0.05$ ) compare to zileuton loaded nano-emulsifying systems in lowering the TBARS ( $1.45 \pm 0.3 \mu\text{M}$ ,  $p < 0.01$ ). The levels of cytokines, namely IL-1 $\beta$ , IL-6, and TNF- $\alpha$  levels in BALF were also quantified (Figure 5B, C, and D). The developed zileuton nano-emulsifying formulation was found to be more effective in reducing expression of inflammatory cytokines when compared to conventional zileuton. This indicates the potential of the developed formulation in reduction of inflammation in acute lung injury. However, when compared to the conventional zileuton formulation ( $1.80 \pm 0.2 \mu\text{M}$ ,  $p < 0.05$ ), the groups treated with



**Figure 5:** The levels of lipid peroxidation and various inflammatory markers in BALF were measured in four groups: NC:Normal, DC:Diseased, STD:Standard treatment with zileuton oral dispersion, and TEST:Zileuton-loaded nano-emulsifying formulation ( $n = 6$ ). The results of TBARS, IL-1, IL-6, and TNF- are shown in Figures 5A–5D. The data is the mean of six determinations, and the error bars represent the standard deviation. \*, \*\*, and \*\*\* indicate significant differences at  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ , respectively.

zileuton loaded nano-emulsifying systems had a more noticeable effect in lowering the TBARS ( $1.45 \pm 0.3 \mu\text{M}$ ,  $p < 0.01$ ). The levels of cytokines, specifically IL-1 $\beta$ , IL-6, and TNF- $\alpha$ , in BALF were also measured (Figure 5B, C, and D). When compared to conventional zileuton, the developed zileuton nano-emulsifying formulation was found to be more effective in reducing the expression of inflammatory cytokines. This demonstrates the formulation's potential for reducing inflammation in acute lung injury.

## CONCLUSION

Increase in pulmonary diseases has triggered the research in inhaled therapeutics in last 5 decades. The interest in inhalation delivery increased not only for the treatment of airways disorders but also for systemic delivery. However, this route also has biological barriers that hinder drug uptake. Therefore, research in particle size has been the main focus to improve permeation and bioavailability. The physicochemical properties and the stability of the drug need to be considered while designing an inhalation product. Additionally, pulmonary mechanics that affects lung deposition of particles also need to be considered.

Inhalation drug delivery gives high local exposure of lungs and also reduces systemic side effects. Zileuton has been used to treat airway diseases and has beneficial effects on pulmonary function and airway inflammation. However, chronic use of conventional zileuton may cause adverse reactions. As a result, a zileuton-loaded nano-emulsifying system was successfully developed to reduce dosage while improving specific targeting. In comparison to other organs, spherical and homogeneous globules demonstrated greater targeted effectiveness and selectivity for lung tissue. Treatment in the acute lung inflammation animal model clearly showed reduced neutrophil accumulation in the lungs when the zileuton was delivered by inhalation route. The results indicate that the prepared zileuton-loaded nano-emulsifying systems has selectivity for the lung tissue and it is possible to get high and effective therapeutic concentrations and pharmacological response. In addition, exploring the new route of administration will give a new lease of life to the molecule. Spontaneously nano-emulsifying systems is a flexible formulation approach. It also has ease of scale up as it needs minimum machineries to manufacture. To increase the solubility and bioavailability of a variety of medications that are poorly soluble, it is easily applied.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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