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Research Article

ASSESSING THE ROLE OF VILANTEROL ON ANTI-INFLAMMATORY MARKERS USING ANIMAL MODELS OF ACUTE AND CHRONIC INFLAMMATION: A COMPREHENSIVE EVALUATION

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ABSTRACT

Objectives: The objective of the study was to evaluate the effect of Vilanterol on inflammatory responses using acute and chronic model of inflammation in Wistar rats.

Methods: Male and female Wistar rats (150–200 g) were acclimatized and assigned into control and treatment groups. In the acute inflammation model, carrageenan 0.1 mL of 1% solution was injected subcutaneously into the left hind paw 60 min after the drug administration. Volume of the paw was monitored at regular intervals up to 24 h using a digital plethysmometer. For chronic inflammation, sterile cotton pellets (50±1 mg) were surgically placed subcutaneously in the scapular region. Animals received daily treatment for further 7 days. Blood samples were collected for biochemical analysis, and pellets were excised to measure wet and dry weights to assess granuloma formation and change in weight.

Results: In the carrageenan-induced paw edema model, a significant reduction in paw volume was observed in the Vilanterol and Salmeterol-treated groups ($p<0.001$). In the chronic model of inflammation, a significant ($p<0.001$) decrease in granuloma weight was noted in animals treated with Vilanterol. Biochemical analysis of blood samples further supported anti-inflammatory action with reductions in proinflammatory markers.

Conclusion: Anti-inflammatory effect of vilanterol was evident in both acute and chronic models. These findings suggest the potential role vilanterol against inflammation, along with its known bronchodilator effects.

Keywords: Vilanterol, Inflammatory, Paw edema, Granuloma, Wistar rats, Bronchodilator.

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) and asthma are debilitating respiratory conditions characterized by airway inflammation and airflow limitation, leading to significant morbidity and mortality worldwide [1,2]. Inflammatory cells responsible for the symptoms, such as T-lymphocytes release mediators, eosinophils, and mast cells, causing bronchoconstriction and mucus hypersecretion. Structural changes like airway remodeling further contribute to disease persistence. Genetic and environmental factors interact to initiate and exacerbate these inflammatory responses [3]. Bronchodilators are the mainstay in the symptomatic treatment of these conditions [4]. Despite advancements in pharmacotherapy, the management of these diseases remains challenging, necessitating the exploration of novel therapeutic strategies [5,6].

While salbutamol, a short-acting β_2-agonist, provides rapid bronchodilation with limited duration and modest anti-inflammatory effects, long-acting β_2-agonists such as salmeterol and vilanterol offer sustained receptor activation, which has been increasingly associated with modulatory effects on inflammatory processes, including the suppression of proinflammatory cytokine release and inflammatory cell recruitment. Emerging evidence suggests that this prolonged β_2-adrenergic activation may confer additional anti-inflammatory benefits beyond bronchodilation, thereby providing a strong rationale for systematically investigating the anti-inflammatory potential of Vilanterol in experimental models of acute and chronic inflammation [7]. There are some limitations with these drugs as well; frequent use of salbutamol may lead to tolerance, reduced efficacy, and potential cardiovascular side effects [8].

Vilanterol is a long-acting β_2-adrenergic agonist (LABA) that has emerged as a promising bronchodilator in the treatment of COPD and asthma [9]. By activating β_2-adrenergic receptors on bronchial smooth muscle cells, Vilanterol promotes bronchodilation, thereby improving airflow and alleviating symptoms of airway obstruction. However, beyond its bronchodilatory effects, the impact of Vilanterol on airway inflammation, both acute and chronic, remains incompletely understood.

Inflammatory processes play a central role in the pathogenesis of COPD and asthma, contributing to airway remodeling, mucus hypersecretion, and exacerbations of disease [10]. Vilanterol showed long-standing relief from the bronchoconstriction compared to the Salmeterol [11]. Therefore, elucidating the effects of Vilanterol on inflammatory markers is crucial for comprehensively understanding its therapeutic potential in these conditions. Animal models, particularly rodents such as Wistar rats, offer valuable tools for investigating the complex interplay between bronchodilation and inflammation in respiratory diseases.

Wistar rats have been extensively used in preclinical studies due to their genetic homogeneity, ease of handling, and physiological similarities to humans. Moreover, the availability of well-characterized inflammatory markers in rats facilitates the assessment of drug-induced changes in acute and chronic inflammation.

In this study, we aimed to evaluate the effect of Vilanterol on acute and chronic inflammatory markers in Wistar rats. We hypothesized that Vilanterol would significantly attenuate inflammation by reducing specific pro-inflammatory markers in both acute and chronic experimental models. By delineating its anti-inflammatory effects across these models, the study seeks to enhance understanding of the underlying therapeutic mechanisms of Vilanterol and to support its clinical relevance in the management of inflammatory airway diseases such as COPD and asthma.

METHOD

Wistar rats of both the sex, weighing 150–200 g were selected for the study. The animals were housed in CCSEA registered animal house with clean, well-ventilated laboratory cages and acclimatized to the experimental conditions before the commencement of the study. They were kept under controlled conditions such as 12 h' light/12 h' dark cycle, an ambient temperature of 23±2°C, and a relative humidity 45–65%. Standard laboratory feed and filtered drinking water (Aqua guard purified) were provided ad libitum to ensure adequate nutrition and hydration. All the animals were monitored regularly to ensure their health and welfare maintained throughout the experimental period. The study started after obtaining the ethical clearance from IAEC (BVDUMC/2215/2024/02/18).

Chemicals and reagents

Vilanterol trifenatate, Salmeterol trifenatate, and Carrageenan (λ-carrageenan) procured from Sigma-Aldrich, US. Ketamine-6iPAIN Healthcare private limited, Delhi, tumor necrosis factor-alpha (TNF-α) enzyme-linked immunosorbent assay (ELISA) kit- KRISHGEN Biosystems, USA, interleukin (IL-6) ELISA kit- KRISHGEN Biosystems, USA.

After acclimatization, animals were randomly assigned to six experimental groups consisting of 8 animals/group using a lottery method, wherein numbered slips corresponding to each group were drawn to ensure unbiased allocation. This process ensured equal group sizes and minimized selection bias.

Vilanterol and Salmeterol were administered at predetermined doses based on previously published pharmacological studies and preliminary dose-optimization experiments [12]. Vilanterol was administered at a dose of 1 mg/kg, while Salmeterol was administered at 1 mg/kg. Both drugs were administered via the oral route once daily. The drug solutions were freshly prepared on the day of experimentation. Vilanterol and Salmeterol were dissolved in normal saline, which served as the vehicle for the control group.

Carrageenan-induced paw edema

In the acute carrageenan-induced paw edema model, animals received a single dose of the test drugs. Vilanterol 1 mg/kg and Salmeterol 1 mg/kg were administered through the oral route 60 min before the subplantar injection of carrageenan. Control animals received an equivalent volume of the vehicle through the same route. Acute inflammation was induced by injecting 0.1 mL of 1% carrageenan suspension into the subplantar region of the right hind paw.

Before drug administration, baseline paw volume of each rat was recorded using a digital plethysmometer (Paw Edema (Plethysmometer) Meter, IITC Life Science, Catalog Number 520, SN.-H11050283, 23924 Victory Blvd. Woodland Hills, California 91367, United States) to establish control readings. Sixty minutes after the administration of the test and standard drug, inflammation was produced with an injection of 0.1 mL of 1% carrageenan solution, freshly prepared in normal saline, into the planter region of the left hind paw subcutaneously. The paw volume was subsequently measured at1 h, 2 h, 3 h, 4 h, 5 h, and 24 h following carrageenan injection using the same digital plethysmometer to assess the progression of edema. For biochemical estimations, blood samples were collected from all the animals after 5 h under ketamine anesthesia from the retro-orbital plexus. All procedures were performed under aseptic precaution to minimize stress and ensure animal welfare.

Cotton pellet-induced granuloma

Cotton pellets weighing 50±1 mg were prepared and sterilized using a hot air sterilizer before implantation. The skin over the scapular region, below the nape of the neck, was carefully shaved and disinfected with 70% ethanol. Each animal was anesthetized with Ketamine in 100 mg/kg dose intraperitoneally to minimize the discomfort during the procedure. A 1 cm incision was made on the left side of the scapular region, and a subcutaneous tunnel was prepared by passing a blunt forceps. A sterilized cotton pellet of 50 mg is used without impregnation with any drug or chemical, serving as inert foreign bodies to elicit a granulomatous inflammatory response, was inserted into the tunnel. The incision was then closed with sterile absorbable Vicryl 3-0 sutures. The same procedure was repeated on the right side to implant another pellet.

Following the pellet implantation, the animals received drug treatment according to their respective experimental groups for a duration of seven consecutive days. On the 8th day, under ketamine anesthesia, blood samples were taken from the retro-orbital plexus. After blood collection, the implanted cotton pellets were separated carefully and removed from both sides, and weight of each pellet was immediately recorded. The pellets were dried in a hot air oven 60±0.5°C until there was no further change in the weight. The dry weight of the Pellets was measured, and the difference between the final dry weight and the initial weight was considered as the net granuloma tissue formation, representing the extent of chronic inflammation.

Markers for acute and chronic inflammation were evaluated from the blood samples using standard biochemical and immunoassay methods.

To assess the acute anti-inflammatory response, serum levels of IL-6, TNF-α, and C-reactive protein (CRP) were measured using ELISA kits, Serum levels of TNF-α (Lot No.: RTNFA0224) Catalog Number-KB3145, IL-6 (Lot No.: RI60124) Catalog Number-KB3068were quantified using commercially available rat-specific ELISA kits procured from KRISHGEN Biosystems, USA All assays were performed strictly according to the manufacturers' instructions.

For the estimation of chronic inflammation and oxidative stress, levels of malondialdehyde (MDA), a marker of lipid peroxidation, and glutathione (GSH) levels were determined from plasma samples. MDA concentration, a marker of lipid peroxidation, was estimated by the thiobarbituric acid reactive substances (TBARS) method. GSH levels were measured by the Ellman's method, based on the reaction of GSH with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to form a yellow-colored complex, which was read at 412 nm. These biochemical estimations provide a comprehensive evaluation of both inflammatory and oxidative stress parameters, thereby enabling assessment of the anti-inflammatory efficacy of the test drugs in both acute and chronic model of inflammation [13,14].

Statistical analysis

The data collected from all experimental observations were compiled and organized using Microsoft Excel for further analysis. Data were expressed as mean±standard error of the mean. Prior to inferential analysis, normality of data distribution was assessed using the Shapiro–Wilk test**.** As the data were found to be normally distributed, one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test to determine the significance of differences between groups was used. $p<0.05$ was considered statistically significant. ALL results were expressed as mean±standard deviation (SD) to represent the variability within each group.

RESULTS

At baseline (0 h), paw volumes before drug treatment across all experimental groups were comparable. In the disease control group, a progressive increase in paw volume was observed, peaking at 5 h, indicating ongoing inflammation. In contrast, groups treated with the test drugs showed a gradual reduction in paw volume following administration. Notably, at 2 h post-treatment, paw volume significantly decreased in the Vilanterol group ($p<0.01$) and the Salmeterol group ($p<0.05$). This reduction became more pronounced at 3 h, with Vilanterol showing highly significant inhibition ($p<0.001$) and Salmeterol also demonstrating a significant effect ($p<0.01$). By 4 h, both drugs exhibited comparable anti-inflammatory effects, which were sustained up to 24 h, indicating prolonged activity.

In the disease control group, the weight of both wet and dry cotton pellets was significantly higher, indicating substantial granuloma formation and chronic inflammation. Treatment with Vilanterol led to a marked reduction in both wet ($p<0.001$) and dry ($p<0.001$) pellet weights, demonstrating significant anti-inflammatory activity. Similarly, Salmeterol treatment also resulted in a significant decrease in wet ($p<0.001$) and dry ($p<0.001$) cotton pellet weights, reduction in cotton pellet weight indicates anti-exudative and anti-proliferative activity, confirming its efficacy in reducing chronic inflammatory responses.

In the disease control group, a marked elevation in proinflammatory cytokines was observed, indicating systemic inflammation. Treatment with Vilanterol significantly reduced the levels of both TNF-α and IL-6 ($p<0.001$), reflecting potent anti-inflammatory activity. Salmeterol also led to a significant decrease in TNF-α levels ($p<0.001$) and also IL-6 levels ($p<0.001$) compared to the disease control group, suggesting a differential modulation of inflammatory pathways.

Effect on oxidative stress markers (MDA and GSH): The control group exhibited significantly elevated MDA levels and reduced GSH levels, indicating pronounced oxidative stress. Treatment with Vilanterol significantly lowered MDA levels ($p<0.001$) and increased GSH levels ($p<0.01$), suggesting a protective antioxidant effect. Similarly, Salmeterol treatment significantly reduced MDA levels ($p<0.001$) and showed a non-significant increase in GSH levels, reflecting a partial amelioration of oxidative damage.

DISCUSSION

Inflammation is a complex biological response to harmful stimuli, involving the activation of immune cells and the release of cytokines, prostaglandins, and reactive oxygen species [15]. Existing options available shows various adverse effects [16]. The present study was conducted to evaluate the anti-inflammatory efficacy of Vilanterol, a LABA receptor, using both acute (carrageenan-induced paw edema) and chronic (cotton pellet-induced granuloma) inflammation models in rats.

The carrageenan-induced paw edema model is one of the most commonly used methods to assess acute inflammation due to its high reproducibility and sensitivity [17]. The inflammation occurs in two distinct phases: the early phase (within 1–2 h), mediated by histamine and serotonin, and the late phase (3–5 h), predominantly mediated by prostaglandins and cytokines such as TNF-α and IL-6 [18]. This model is widely accepted for its reproducibility and sensitivity in detecting anti-inflammatory activity, especially during the early phase of inflammation, which involves the activation of the complement system and the release of histamine, serotonin, bradykinin, and prostaglandins due to the presence of sulfated polysaccharides in carrageenan [19].

Vilanterol significantly reduced paw edema at various time points, with a more pronounced effect observed during the later phase of inflammation (Fig. 1). This suggests that Vilanterol exerts its anti-inflammatory action by modulating the cytokine-mediated phase of the inflammatory response, which typically involves the recruitment and activation of immune cells and the release of pro-inflammatory mediators such as TNF-α, IL-1β, and IL-6. The attenuation of edema during this phase indicates that Vilanterol may interfere with the signaling pathways regulating cytokine production or the downstream vascular permeability changes induced by these mediators.

These findings are consistent with the study by Jude *et al*. [20], who demonstrated the anti-inflammatory role of β2-agonists in airway inflammation by reducing cytokine levels and inflammatory cell infiltration. Their study demonstrated that β_2-agonists reduce cytokine release and inhibit inflammatory cell infiltration in airway tissues, thereby contributing to the overall suppression of inflammatory responses [21]. Collectively, the present findings reinforce the evidence that Vilanterol, through β_2-receptor activation, may downregulate key pro-inflammatory pathways, ultimately mitigating both local tissue edema and cellular inflammation.

The cotton pellet-induced granuloma model is a well-established method for evaluating chronic inflammation, particularly the proliferative phase involving fibroblast activation, collagen deposition, and exudate formation [22]. Vilanterol significantly reduced both wet and dry pellet weights (Fig. 2), suggesting its ability to inhibit not only transudate fluid accumulation but also granulomatous tissue proliferation. These observations are supported by previous findings on Salmeterol, another LABA, which showed similar reductions in inflammatory responses in COPD models [23].

In biochemical analysis, it showed reduced levels of TNF-α and IL-6 (Table 1), particularly in the Vilanterol-treated group. TNF-α is a central mediator in both acute and chronic inflammation, and its suppression reflects the drug's systemic anti-inflammatory potential. Interestingly, while both Vilanterol and Salmeterol decreased TNF-α, only Vilanterol significantly reduced IL-6, indicating a differential cytokine modulatory effect between the two drugs. These findings are supported by Barnes (2002), who highlighted the ability of β2-agonists to suppress NF-κB activation, a key transcription factor regulating the expression of proinflammatory cytokines [24].

Furthermore, oxidative stress markers such as MDA and GSH were also measured to assess the chronic inflammatory response. Elevated MDA and decreased GSH levels in the disease control group indicated high oxidative stress. Treatment with Vilanterol significantly decreased MDA and increased GSH levels, suggesting antioxidant potential in addition to its anti-inflammatory effects (Fig. 3). Similar antioxidant activity has been reported for β2-agonists, as they are known to reduce oxidative burden through both direct and indirect mechanisms [25,26]. The dual anti-inflammatory and antioxidant effects of Vilanterol may be

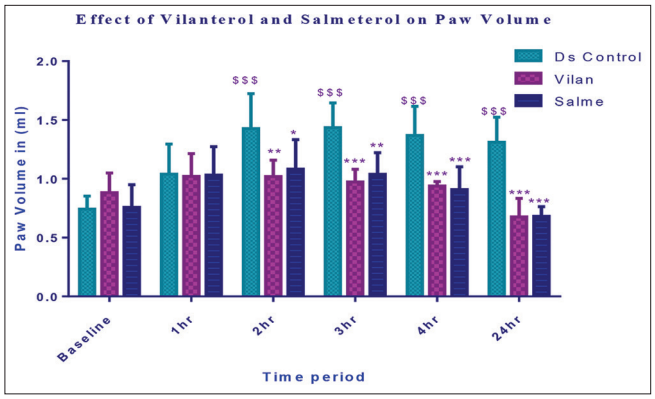

Fig. 1: Effect of vilanterol on paw edema in Wistar rats

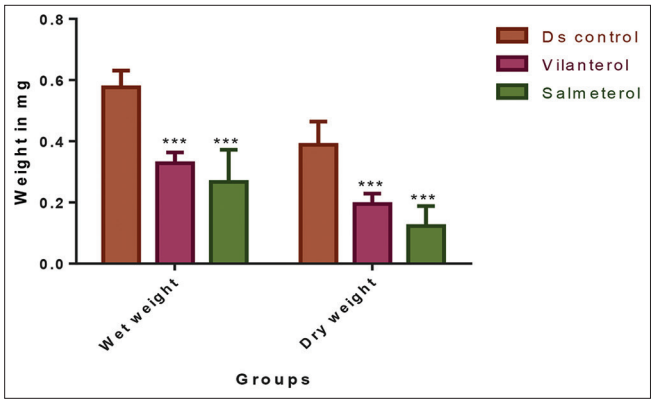

Fig. 2: Effect of vilanterol on pellet weight in Wistar rats. In the chronic inflammation model- Cotton pellet induced granuloma model. Values are expressed as mean±SD; n=8; One-way ANOVA followed by Tukey's test. *p<0.05,p<0.01, ***p<0.001 in comparison with disease control**

Table 1: Effect of vilanterol and salmeterol on serum TNF-α and IL-6 levels

Groups/inflammatory parameters	TNF-α (pg/mL)	IL-6 (pg/mL)
Control	44.47±3.35	77.39±9.53
Vilanterol	29.19±6.55***	53.60±12.39**
Salmeterol	28.91±5.96***	48.25±9.63***

Values are expressed as mean±standard deviation; n=8; One-way analysis of variance followed by Tukey's test. **p<0.01, ***p<0.001 in comparison with disease control. TNF: Tumor necrosis factor-alpha, IL-6: Interleukin-6

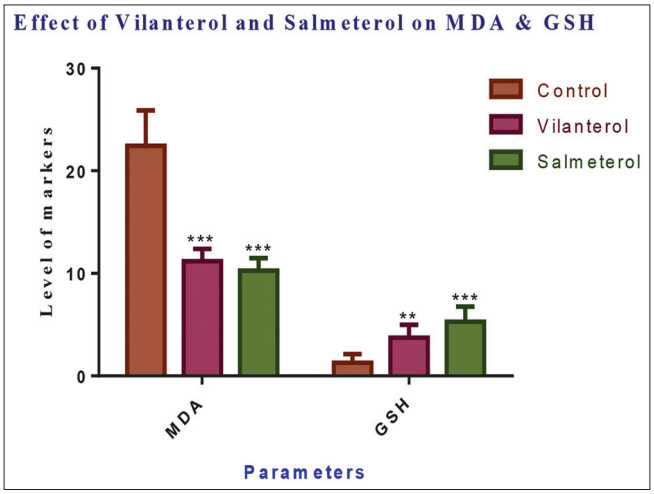

Fig. 3: Effect of vilanterol and salmeterol on serum interleukin-6 levels

attributed to its long-acting β2-agonist properties, which not only relax bronchial smooth muscle but also modulate inflammatory pathways. The suppression of cytokine production and reduction in oxidative stress collectively contribute to its therapeutic benefits beyond bronchodilation.

CONCLUSION

The current study supports the broad therapeutic potential of Vilanterol, demonstrating significant anti-inflammatory and antioxidant effects in both acute and chronic models of inflammation. These findings align with and extend the existing literature on LABAs, suggesting their role not only in symptomatic relief but also in modulating underlying inflammatory mechanisms, making them promising candidates for the management of chronic inflammatory diseases such as asthma, COPD, and possibly other systemic inflammatory conditions. The results suggest that Vilanterol exerts significant anti-inflammatory effects across both phases of inflammation.

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AUTHORS' CONTRIBUTION

Author 1 conducted the experimental work, collected data, performed the statistical analysis, validation of results, and technical support.

Author 2 conceptualized and designed the study, supervised the research work, and critically revised the manuscript and approved the final version.

CONFLICTS OF INTEREST

Nil.

ETHICAL ISSUES

None.

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