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Unveiling Potent Anti-Asthmatic Effect of Curcumin in Combination with Salmeterol in Swiss Albino Mice

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Asthma is a long-term inflammatory respiratory condition marked by alterations in the airways and an increase in inflammatory cell infiltration. It has been observed that Curcumin possesses immune-modulating, anti-inflammatory, and relaxing properties for smooth muscle in the airways. Salmeterol is believed to ease the smooth muscles of the airways.
Objective: Swiss Albino mice were used in the research to examine the combination anti-asthmatic effects of Curcumin and Salmeterol in asthma produced by ova albumin and milk induced eosinophilia and leucocytosis.

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Methods: The mice received pre-treatment with Curcumin (10 mg/kg, 20 mg/kg intraperitoneally) as well as Salmeterol (5 mg/kg) after being stimulated with an Ovalbumin (OVA) challenge and milk. After the induction period, various hematological, biochemical, molecular (ELISA), and histological analyses were performed.
Results: The findings demonstrated that the combined treatment decreased the animal's overall leukocyte and eosinophil numbers in a manner that was dose-dependent. Additionally, the therapy reduced albumin and overall protein amount in serum, BALF and lung tissues, facilitated changes in haematological parameters, and reduced the rise of Th2 cytokines (IL-4, TNF-α, IL-13) levels that is induced by OVA in lungs and BALF, total IgE level in serum. The combined action of Curcumin and Salmeterol reduced OVA-induced inflammatory influx and ultrastructural abnormalities, according to histopathological evaluation.
Conclusion: The findings of this investigation demonstrate that curcumin and salmeterol together possess anti-asthmatic effects through suppressing Th2 triggered immune response and possessing an anti-inflammatory effect and anti-allergic effect. Thus combination of treatments might be a novel technique for managing asthma.

Keywords: Asthma; ovalbumin; curcumin; salmeterol; IL's; TNF-α; leucocytosis; eosinophilia.

1. INTRODUCTION

Asthma is a chronic inflammatory and incurable condition of the lungs [1]. It is a long-term airways condition marked with recurrent episodes of wheezing, dyspnoea, and constricted sensation throughout the chest area [2]. Long-term airways infection can be related to a hyperbolic activity of the airways to certain stimuli, including infectious agents, allergenic substances, and physical activity. This results in recurring bouts of dyspnoea, tightening of the chest, and coughing, which can fluctuate with duration and severity as the time passes [3].

According to the World Health Organization's statistics of 2004, asthma constituted a major source of illness and death in Indian villages, accounting for 57,000 deaths throughout the country [2]. According to data from WHO, asthma affected 262 million people globally in the year 2019 and remained the third-largest prevalent reason for hospitalizations among adolescents under the age of fifteen years [4]. It is predicted that 400 million individuals worldwide might suffer from asthma by 2025 as an outcome of urbanization [5].

The major symptoms include fluctuating expiration airflow limits, remodelling, and rapid responses of the airways [6]. The release of inbuilt and internal agents upon responses to allergies and airway inflammation, such as histamine, leukotrienes (LTs), bradykinin, prostaglandins (PGs), nitric oxide, platelet activating factors (PAF), chemokines, and endothelin via mast cells, might be the cause of such manifestations [7]. A variety of inflammation-associated agents, including mast cells, eosinophils, T lymphocytes, and dendritic cells are released into the respiratory tract during the disease infiltrate. These agents are crucial for the occurrence of asthma [8]. For the purpose of managing and treating asthma effectively, several therapy approaches have been developed. Amongst them are leukotriene modifiers, β2 adrenergic agonists, corticosteroids, mast cell stabilizing agents, inhaled bronchoprovocation complications, as well as anticholinergic and antihistaminic drugs. At present, combined inhalation devices that comprise a corticosteroid and a long-acting β - agonist constitute an extremely successful therapy for asthma. While the precise mode of effectiveness of the combined treatment remains unclear, there is ample evidence of the synergistic effects between those two medication groups [9,10]. Individuals who use various corticosteroids for extended periods of time develop a variety of complications like diabetes, fractures, pneumonia, gastrointestinal disease, glaucoma and hyperlipidaemia [11]. Therefore, its critical to choose a replacement that will either completely eliminate or significantly reduce the adverse consequences of corticosteroids.

The family Zingiberaceae includes *Curcuma Longa*, whose rhizome yield Curcumin, a polyphenol that is associated with immune-modulating, anti-inflammatory, and smooth muscles of the airways soothing properties. Curcumin has been shown to be beneficial in the management of respiratory-related issues [12,13]. Salmeterol is a specific long-acting β - agonist that induces bronchodilation by relaxing the airway smooth muscles and preventing the

ejection of allergic mediators from mast cells [14]. Curcumin, when combined with salmeterol, may be a promising medication to replace corticosteroids. In this research, Swiss Albino mice are used to examine the combining impact of Salmeterol and Curcumin on asthma.

2. MATERIALS AND METHODS

2.1 Chemicals

The supplier of Curcumin was YUCCA Enterprises, located in Mumbai, India. The commercial formulations of Salmeterol (Serobid rotacaps) and Dexamethasone sodium phosphate (Dexona injectable) were used. Cow Milk was purchased from local dairy. Himedia supplied the Ovalbumin (OVA, egg albumin grade II) and SDFCL Fine-Chem supplied aluminium hydroxide, respectively.

2.2 Dose Selection

According to the literature survey the doses of the required drugs was Dexamethasone (50 mg/kg) [15] and (2 mg/kg) [16] *i.p*, Salmeterol (5mg/kg) *i.p* [17] and Curcumin (10 mg/kg), (20mg/kg) *i.p* [18].

2.3 Experimental Animals

Krupanidhi College of Pharmacy, Bangalore, India provided Swiss Albino female mice weighed between twenty-five and thirty grams. To accommodate and adapt them, housing for animals having adequate air circulation was used. A week before the start of the study, the lab surroundings were kept in compliance with the requirements set by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The aforementioned comprised a 25 ± 2°C regulated temperature as well as 50–60% relative humidity. Water and food were supplied to the animals [15,19]. The Institutional Ethics Committee accepted the research protocols, which was assigned with the number **KCP/IAEC/PCOL/134/AUG-2023.**

2.4 Experimental Protocol

2.4.1 Milk-Induced leucocytosis and eosinophilia in mice

Five different groups comprising of Swiss Albino female mice were chosen at random. The animals of group I were given a vehicle (distilled water 10 ml/kg *p.o*.) alone and shall behave as regular controls. The animals grouped into II, III, IV, and V received subcutaneously the cool milk after boiling it for twenty minutes at seventy degrees Celsius. Group III animals received standard intraperitoneal (*i.p*.) administration of Dexamethasone (fifty milligrams per kilogram) and Salmeterol (five milligrams per kilograms) [15,17]. Thirty minutes prior to milk administration, Group IV and V were administered two distinct dosages of salmeterol and curcumin via intraperitoneal injection (ten milligrams per kilograms + five milligrams per kilogram) and (twenty milligrams per kilograms + five milligrams per kilograms), accordingly [17,18]. A minimal diethyl ether anaesthesia was administered during the retro orbital plexus blood sample gathering procedure to determine the total leukocytes and eosinophils [15].

2.4.2 Determination of total leukocyte and eosinophil count

Overall leukocytes and eosinophils levels were determined in every single group twenty-four hours after the introduction of milk and prior to the test substance treatment. The variation between the prior to and post twenty-four hours treatment counts for total leukocytes and eosinophils was measured [15].

2.4.3 Ovalbumin-induced airway model of asthma

OVA was used for challenging and sensitizing the mice. In summary, mice had been sensitized intraperitoneally on day zero with 0.2 mL of (PBS, pH 7.4) comprising twenty µg OVA and two mg of aluminium hydroxide gel. On days seven, fourteen, and twenty-one the sensitization process was performed again. One hour following the administration of drug on days 24-27, the mice received daily intranasal inhalation of 100 µg OVA in an amount of 50 µl PBS, with the exception of the normal control group. Following a similar procedure, normal control mice received sensitization and was challenged with PBS buffer separately [16].

Swiss Albino female mice were divided into five groups, with six mice in each group. Group 1 consisted of untreated, normal mice (normal control group). Groups 2 to 5 were exposed to OVA in the manner previously mentioned. No medication was given to Group 2, which is the positive control group. Group 3 received

treatment with two doses of salmeterol (five milligrams per kilograms) and DEX (two milligrams per kilograms) [16,17] which is the standard group. The amount of curcumin + salmeterol administered to groups 4 i.e. (Treatment 1) and 5 i.e (Treatment 2) were (ten milligrams per kilograms) + (five milligrams per kilograms) and (twenty milligrams per kilograms) + (five milligrams per kilograms) respectively [17,18]. Between day 21 to 27, all medications were administered intraperitoneally (*i.p*.) [16].

2.4.4 Physical characteristics

Following 27-days medication regimen, physical characteristics such as body weight and relative lung weight were assessed [20].

2.4.5 Estimation of cell count in blood

Leukocyte counts were conducted on each animal after the blood was drawn under anaesthesia and placed into a heparinized tube. These samples were taken via the mice retro-orbital plexus. After centrifuging all the samples for ten minutes at four degree Celsius at 500 × g, the pellet cells were rinsed in 0.5 mL of saline, and the total amount of cells was determined using an automatic cell counter. To carry out a differential analysis, just a small amount of the blood cells was transferred upon slides along with staining by Field's dye for identifying neutrophils, lymphocytes, or eosinophils utilizing common morphologic markers [4].

2.4.6 Collection of Broncho alveolar Lavage Fluid (BALF)

Following the anaesthesia of the mice, the tracheas were intubated and two 0.8 ml samples of cold PBS were used to rinse them. After collecting, the specimens of Broncho Alveolar Lavage Fluid (BALF) they were promptly centrifuged. Before being employed for the cytokines experiment, the supernatants were kept at -80 °C until it was used. Meanwhile, the cell pellets were placed back in PBS to determine total and differential cells. The haemocytometer was used to quantify the total amount of cells. The Kwik-Diff staining kit by (Kaushik laboratory and clinic, Bangalore) was utilized for determining the eosinophils, neutrophils, macrophages, and lymphocytes in BALF on cytospin preparation. Each slide comprised of 200 cells [21].

2.4.7 Lung tissue homogenate preparation

The animals were executed after the BAL fluid was collected. A volume of approximately one gram of lung tissue was homogenized using two millilitres of a phosphate-buffered saline, in a 1:2 (w/v) proportion; two millilitres of pH 7.4 PBS was combined with single gram of tissue. The homogenized samples were placed in a refrigerated centrifuge (Eltrec, India) and centrifuged at 10,000 x g for fifteen minutes at forty degrees Celsius. After being separated into small amounts and kept at twenty hundred degrees Celsius, the supernatants were utilized for more analysis [22].

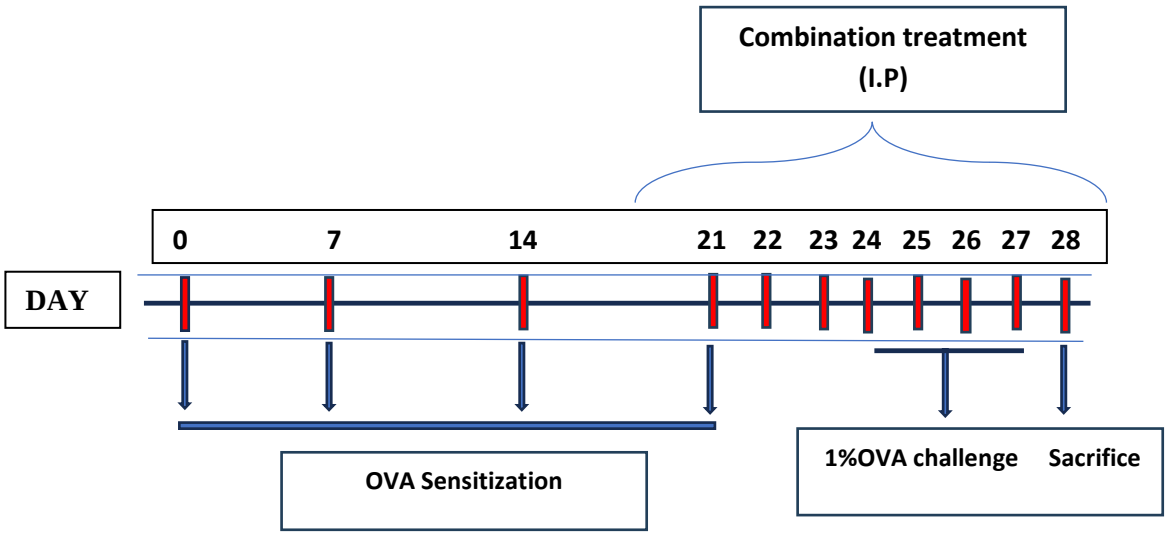


Fig. 1. An inflamed airway model in mice and treatment with curcumin + salmeterol

2.4.8 Evaluation of inflammatory cytokines and serum total IgE level via Enzyme-Linked Immunosorbent Assay (ELISA)

Twenty-four hours following the last OVA challenge, specimens were taken to measure the amounts of cytokines in vivo, BALF and lung homogenate, and overall IgE in serum. Spectrophotometric Analysis ELISA (Kaushik laboratory and clinic, Bangalore) were performed to assess IL-4, IL-13, overall IgE, and tumour necrosis factor-alpha (TNF-α) [10].

2.4.9 Estimation of biochemical parameters

For ten minutes, centrifugation of blood was done at 6000 rpm. Before being analyzed, specimens of serum were gathered and frozen at -800^{0}C. Using easily accessible commercial reagent kits from (ERBA Diagnostics, India) the function of albumin in serum, BALF, and lungs was determined [10].

2.5 Histopathological Examination

Following euthanasia at the last stage of the study, the lungs from mice of all groups were removed, cleansed and rinsed with 0.9% saline, preserved in ten percent neutral formalin, and stained with Haematoxylin and Eosin (H & E) for histological analysis. After trimming, these tissues underwent standard processing. The tissue was processed by immersing it in paraffin wax, cleaning it in xylene, and then dehydrating it in increasing alcohol grades. Blocks of tissue fixed in paraffin wax were sectioned using a Rotary Microtome to a thickness of 4-5 µm. The Haematoxylin and Eosin (H&E) stain was applied to each and every slide. Using the microscope, the slide specimens were inspected, and 100x photographs were obtained to look for inflammatory infiltrations [22,23].

2.6 Statistical Analysis

Results were presented in the form of mean ± SEM. The data set was evaluated using a one-way Analysis of Variance and Dunnet test. The P value of <0.05 was declared significant by graph pad prism.

3. RESULTS

3.1 Milk-Induced Leucocytosis and Eosinophilia in Mice

Leukocyte and eosinophil counts were considerably elevated twenty-four hours after milk treatment at a subcutaneous dose of 4 ml/kg. Comparing the positive control group with the normal control, the positive control group experienced the greatest rise, while treatment 1 (Curcumin 10 mg/kg + Salmeterol 5mg/kg) and treatment 2 (Curcumin 20 mg/kg + Salmeterol 5mg/kg) dramatically reduced the amount of milk-induced leucocytosis and eosinophilia twenty-four hours following therapy as demonstrated in the Table 1 and Figs. 2 and 3.

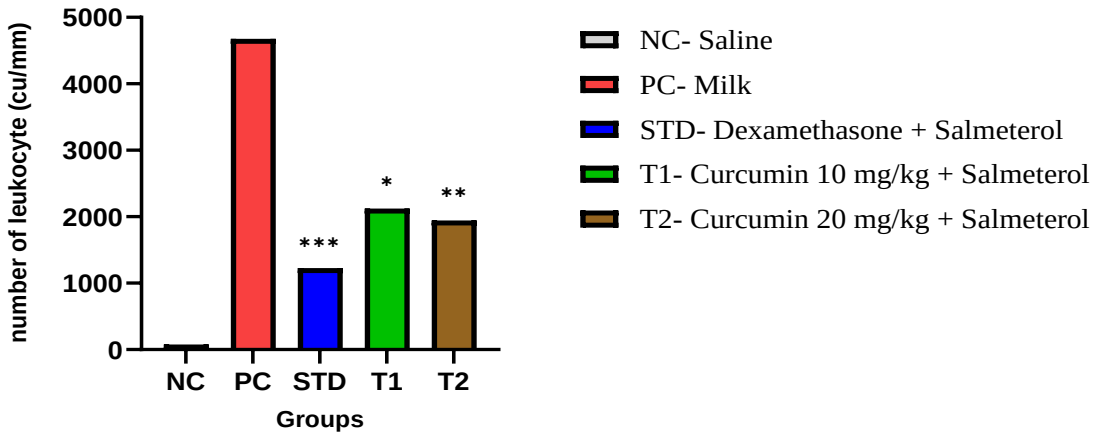


Fig. 2. Impact of curcumin and salmeterol combination milk-induced leukocytosis

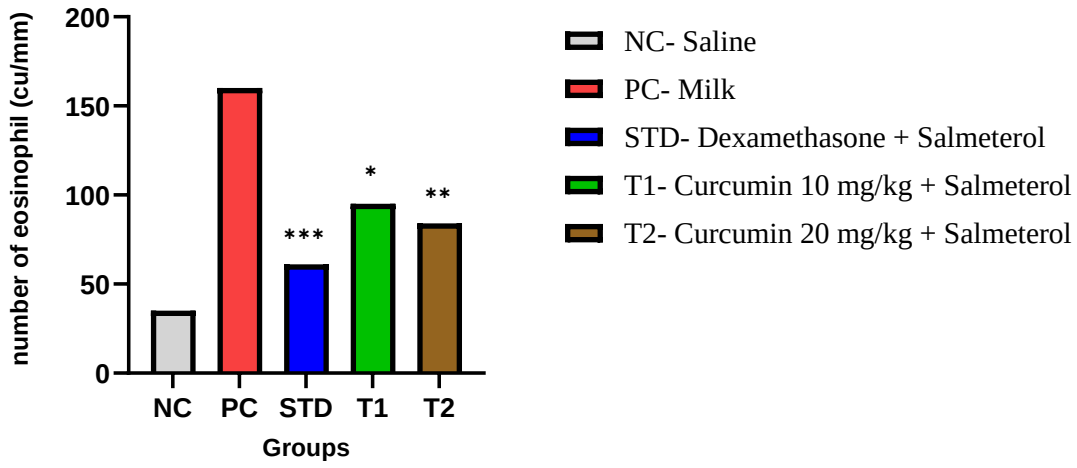


Fig. 3. Impact of curcumin and salmeterol combination milk-induced eosinophilia

Table 1. Impact of curcumin and salmeterol combination milk-induced leukocytosis and eosinophilia in mice

Groups	Difference in no. of leukocyte (cu/mm)	Difference in no. of eosinophil (cu/mm)
NORMAL CONTROL: Saline 10 ml/kg *p.o*	75.00 ± 2.28	34.66 ± 3.77
POSITIVE CONTROL: (Milk 4ml/kg) *s.c*	4678 ± 159.76	160.50 ± 4.37
STANDARD: (Dexamethasone 50 mg/kg + Salmeterol 5mg/kg) *i.p*	1226.83 ± 54.38***	61.00 ± 2.60***
TREATMENT 1: (Curcumin 10 mg/kg + Salmeterol 5mg/kg) *i.p*	2122.00 ± 55.12*	95.00 ± 2.60*
TREATMENT 2: (Curcumin 20 mg/kg + Salmeterol 5mg/kg) *i.p*	1943.83 ± 99.85**	84.16 ± 3.31**

*The result was evaluated using a one-way analysis of variance (ANOVA), following Dennett's Multiple Comparisons Test was then performed. The results are given as Mean ± S.E.M. (n = 6). A Positive Control Group was used to compare each group. non-significant, or ns-. **significant when compared to the Positive Control Group at P<0.05. NC stands for Normal Control. Positive Control (PC). STD: Standard group. T_1: treatment 1 median dose of curcumin T_2: Treatment 2 high dose of Curcumin*

3.2 Ovalbumin-Induced Airway Model of Asthma

3.2.1 Impact of drug combination on changes in total body weight as well as relative lungs weight produced by OVA

On day twenty-eight of the experiment measurements of body weight revealed a decrease in the positive control group and in the standard group, however, a substantial increase was noted in the two treatment groups as shown in Table 2. The relative lung weight of sacrificed mice was discovered to be greatly raised by OVA in the investigation however, as indicated in the Table 2, this rise was significantly averted in the standard group, treatment 1 and 2 shown Table 2.

3.2.2 Impact of drug combination on changes in haematological parameters produced by OVA

While comparing positive control mice to normal control mice, the investigation revealed notable differences in hematologic markers.

Haemoglobin, the number of red blood cells, and PCV% reduced in the positive control group and raised in case of standard group and treatment combinations as shown in Table 3. On the other hand, the WBC, neutrophil, lymphocyte, and monocyte counts were higher in the positive control group. The Table 3 demonstrate that treatment groups exhibited a significant suppression of differential WBCs, neutrophils, and lymphocyte counts.

3.2.3 The impact of a combination of treatments on the OVA-induced changes in differentiated cell counts and inflammation in BALF

As seen in the Table 4, the standard and treatment combination groups demonstrated a substantial reduction in BAL fluid number while the positive control group demonstrated an enormous spike in BALF differential and total number of cells.

3.2.4 Impact of drug combination on variations in Albumin levels and total protein levels in serum and BALF induced by OVA

On comparison with the normal control group, the positive control groups serum and BALF albumin levels were discovered being greater. Compared with the group under positive control, the standard group and treatment combination groups displayed lower serum and BALF albumin level in Table 5.

3.2.5 Impact of drug combination on OVA-induced changes in serum total IgE

ELISA was used to determine the serum's total IgE concentration. According to the results of the research, the positive control group receiving ova treatment had a considerably higher serum concentration of total IgE. Standard group and treatment combinations exhibited a decrease in serum total IgE concentrations as displayed in the Fig. 4.

3.2.6 Impact of drug combination on OVA-induced changes in lung tissue and BALF cytokine examination

Elevations in cytokines associated to the Th2 reaction such as TNF-α and ILs, indicate an effective development of asthma. In this investigation, lung tissue and (BALF) showed increased numbers of cytokines (IL-4, IL-13, and TNF-α) in the positive control group on comparison to the normal control group. In comparison to the positive control group, the standard and treatment groups prevented the increase of IL-13 and IL-4 concentrations in lung tissues as shown in Figs. 8, 9. and BALF as shown in Figs. 5, 6. Figs. 7, 10 indicate that TNF-α concentrations were lower in the treatment as well as standard groups in comparison to the BALF positive control group and Lung tissues.

Table 2. Impact of drug combination on changes in total body weight as well as relative lungs weight produced by OVA

Parameters	Normal control	Positive control (OVA albumin)	Standard Group Dexamethasone (2 mg/kg) + Salmeterol (5mg/kg)	Treatment 1 Curcumin (10 mg/kg) + Salmeterol (5mg/kg)	Treatment 2 Curcumin (20 mg/kg) + Salmeterol (5mg/kg)
Body weight (gm)	26.5±0.54	17.33±0.51	15.33±1.36*	18.66±2.16**	20.60±0.51***
Relative lung weight (%)	0.56±0.14	1.70±0.31	1.04±0.23***	1.39±0.29**	1.16±0.19**

*The result was evaluated using a one-way analysis of variance (ANOVA), following Dennett's Multiple Comparisons Test was then performed. The results are given as Mean ± S.E.M. (n = 6). A Positive Control Group was used to compare each group. non-significant, or ns-. **significant when compared to the Positive Control Group at P<0.05. NC stands for Normal Control. Positive Control (PC). STD: Standard group. T_1: treatment 1 median dose of curcumin T_2: Treatment 2 high dose of Curcumin*

Table 3. Impact of drug combination on changes in haematological parameters produced by OVA

Parameter	Normal control	Positive control (OVA albumin)	Standard Group Dexamethasone (2 mg/kg) + Salmeterol (5mg/kg)	Treatment 1 Curcumin (10 Mg/kg) + Salmeterol (5mg/kg)	Treatment 2 Curcumin (20 Mg/kg) + Salmeterol (5mg/kg)
PCV (%)	45.71±0.29	30.42±0.12	41.39±0.31***	32.28±0.37*	37.60±0.28**
Haemoglobin (mg/dl)	14.52±0.29	9.71±0.16	12.63±0.10***	10.45±0.14*	11.27±0.18**
RBC (x10^6)	5.90±0.17	4.28±0.10	5.33±0.16***	4.16±0.08*	4.57±0.17**
WBC(x10^3)	9.20±0.11	15.45±0.16	10.87±0.13***	13.95±0.08*	12.62±0.18**
Neutrophil (Polymorphs) (%)	8.17±0.11	12.92±0.14	8.84±0.10***	10.32±0.09*	9.53±0.16**
Lymphocytes (%)	81.73±0.17	87.92±0.12	82.33±0.16***	85.14±0.12*	84.37±0.14**
Monocytes (%)	3.06±0.10	4.47±0.18	3.10±0.14***	3.63±0.13*	3.27±0.17**
Eosinophils (%)	2.73±0.11	4.93±0.13	3.04±0.10***	4.01±0.14*	3.54±0.16**

*The result was evaluated using a one-way analysis of variance (ANOVA), following Dennett's Multiple Comparisons Test was then performed. The results are given as Mean ± S.E.M. (n = 6). A Positive Control Group was used to compare each group. non-significant, or ns-. **significant when compared to the Positive Control Group at P<0.05. NC stands for Normal Control. Positive Control (PC). STD: Standard group. $T_{1:}$ treatment 1 median dose of curcumin $T_{2:}$ Treatment 2 high dose of Curcumin*

Table 4. Impact of a combination of treatments on the OVA-induced changes in differentiated cell counts and inflammation in BALF

Parameter	Normal control	Positive control (OVA albumin)	Standard group Dexamethasone (2mg/kg) + Salmeterol (5mg/kg)	Treatment 1 Curcumin (10 mg/kg) + Salmeterol (5mg/kg)	Treatment 2 Curcumin (20 mg/kg) + Salmeterol (5mg/kg)
BALF Total cell count	12.65±0.25	44.61±0.26	19.48±0.13***	28.43±0.24*	23.55±0.16**
Neutrophils (1×10^4cells/ml)	1.88±0.12	2.38±0.15	1.90±0.12***	2.20±0.15*	2.12±0.13**
Eosinophils (1×10^4cells/ml)	8.55±0.12	54.59±0.15	14.00±0.16***	28.54±0.10*	21.55±0.13**
Basophils (1×10^4cells/ml)	7.00±0.33	23.56±0.12	10.60±0.26***	17.91±0.38*	14.57±0.12**
Lymphocytes (1×10^4cells/ml)	15.63±0.25	45.54±0.23	19.06±0.12***	35.58±0.27*	28.59±0.22**
Macrophages (1×10^4cells/ml)	11.01±0.11	23.54±0.26	14.00±0.15***	20.58±0.21*	18.57±0.19**
Epithelial Cells (1×10^4cells/ml)	3.58±0.21	15.80±0.16	4.53±0.25***	8.55±0.27*	6.61±0.21**

*The result was evaluated using a one-way analysis of variance (ANOVA), following Dennett's Multiple Comparisons Test was then performed. The results are given as Mean ± S.E.M. (n = 6). A Positive Control Group was used to compare each group. non-significant, or ns-. **significant when compared to the Positive Control Group at P<0.05. NC stands for Normal Control. Positive Control (PC). STD: Standard group. $T_{1:}$ treatment 1 median dose of curcumin $T_{2:}$ Treatment 2 high dose of Curcumin*

Table 5. Impact of drug combination on variations in albumin levels and total protein levels in serum and BALF induced by OVA

Parameter	Normal control	Positive control (OVA albumin)	Standard group Dexamethasone (2mg/kg) + Salmeterol (5mg/kg)	Treatment 1 Curcumin (10 mg/kg) + Salmeterol (5mg/kg)	Treatment 2 Curcumin (20 mg/kg) + Salmeterol (5mg/kg)
Total protein in Serum (gm/dl)	9.23±0.21	13.96±0.14	10.36±0.12***	12.85±0.13*	11.54±0.21**
Total albumin in Serum (gm/dl)	1.13±0.10	2.55±0.22	1.55±0.19***	2.16±0.15*	1.88±0.18**
Total protein in the lungs (gm/dl)	3.27±0.16	5.17±0.19	3.93±0.18***	4.74±0.24*	4.32±0.18**
Total protein in BALF (gm/dl	1.32±0.19	2.47±0.12	1.84±0.15***	2.20±0.04*	2.02±0.03**
BALF albumin (gm/dl)	0.64±0.03	1.97±0.10	0.94±0.05***	1.63±0.03*	1.34±0.07**

*The result was evaluated using a one-way analysis of variance (ANOVA), following Dennett's Multiple Comparisons Test was then performed. The results are given as Mean ± S.E.M. (n = 6). A Positive Control Group was used to compare each group. non-significant, or ns-. **significant when compared to the Positive Control Group at P<0.05. NC stands for Normal Control. Positive Control (PC). STD: Standard group. $T_{1:}$ treatment 1 median dose of curcumin $T_{2:}$ Treatment 2 high dose of Curcumin*

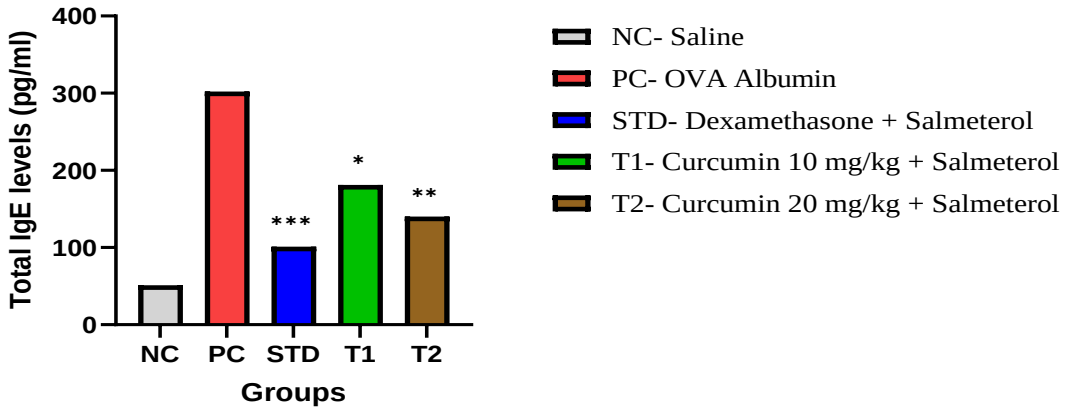


Fig. 4. Impact of curcumin and Salmeterol combination in serum total IgE

*The result was evaluated using a one-way analysis of variance (ANOVA), following Dennett's Multiple Comparisons Test was then performed. The results are given as Mean ± S.E.M. (n = 6). A Positive Control Group was used to compare each group. non-significant, or ns-. **significant when compared to the Positive Control Group at P<0.05. NC stands for Normal Control. Positive Control (PC). STD: Standard group. $T_{1:}$ treatment 1 median dose of curcumin $T_{2:}$ Treatment 2 high dose of curcumin*

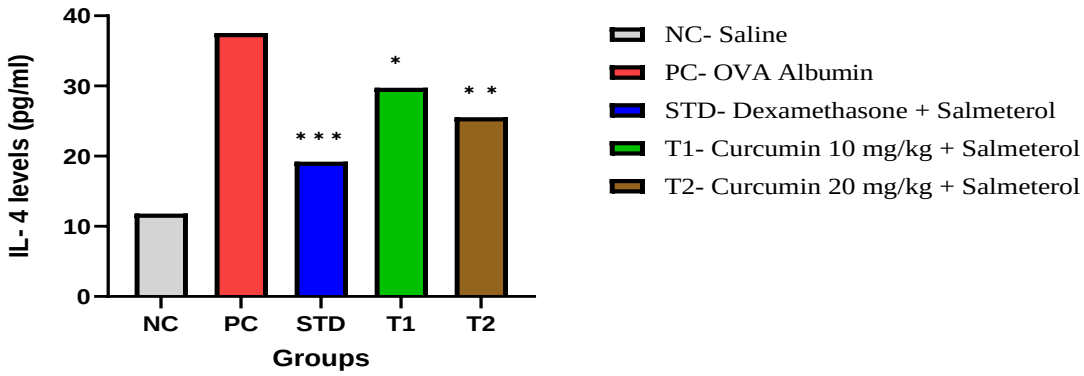


Fig. 5

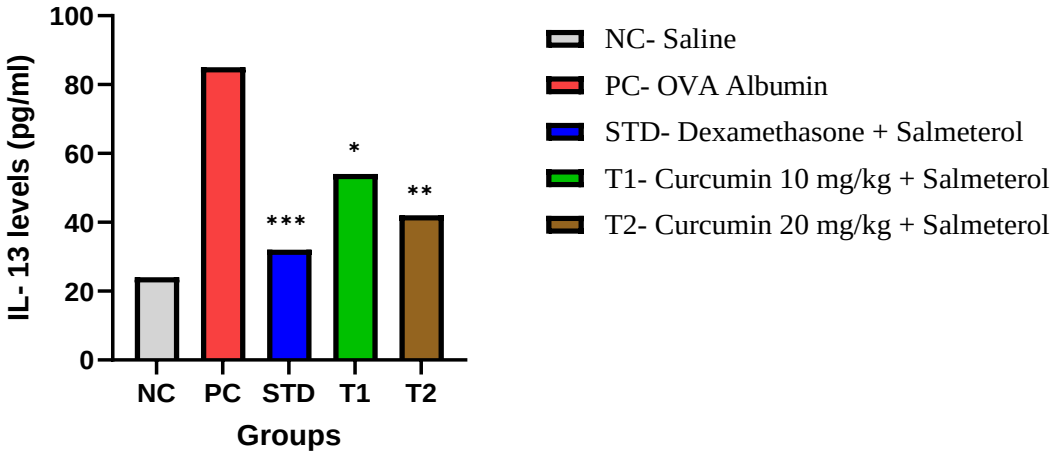


Fig. 6

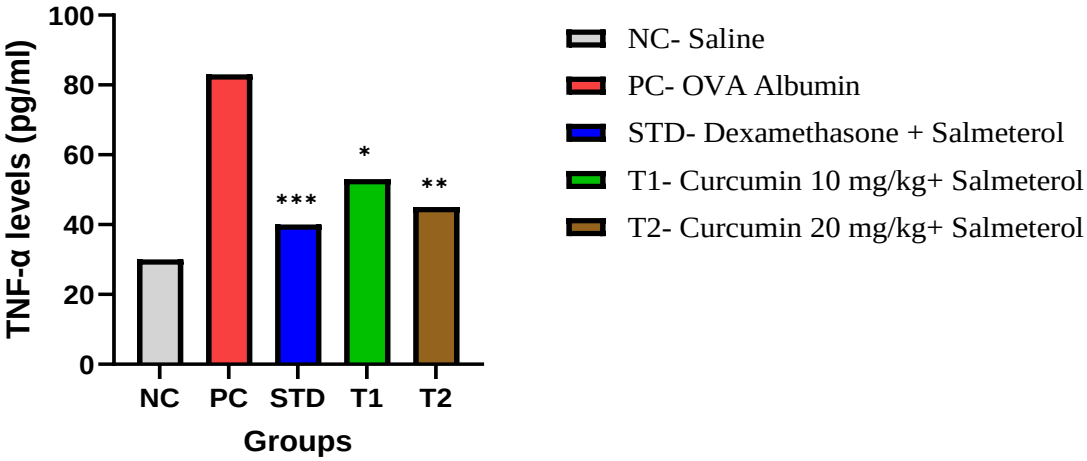


Fig. 7

Figs. 5,6,7. Represents -Impact of Curcumin and Salmeterol combination on IL-4, IL-13, TNF-α in BALF

*The result was evaluated using a one-way analysis of variance (ANOVA), following Dennett's Multiple Comparisons Test was then performed. The results are given as Mean ± S.E.M. (n = 6). A Positive Control Group was used to compare each group. non-significant, or ns-. **significant when compared to the Positive Control Group at P<0.05. NC stands for Normal Control. Positive Control (PC). STD: Standard group. T_1: treatment 1 median dose of curcumin T_2: Treatment 2 high dose of Curcumin*

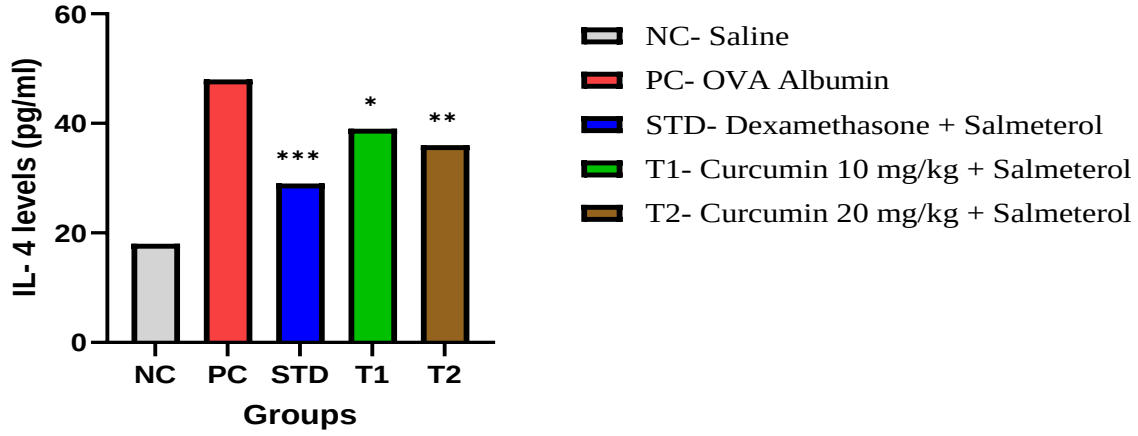


Fig. 8

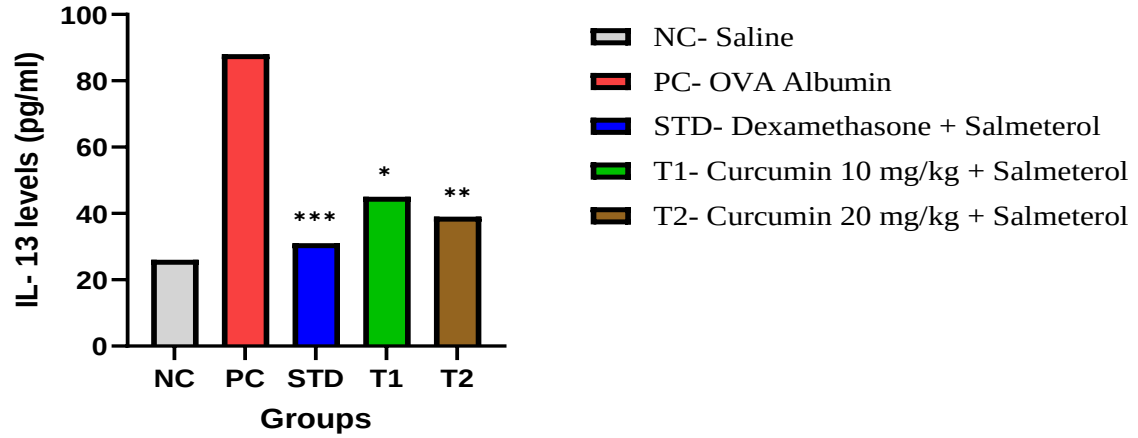


Fig. 9

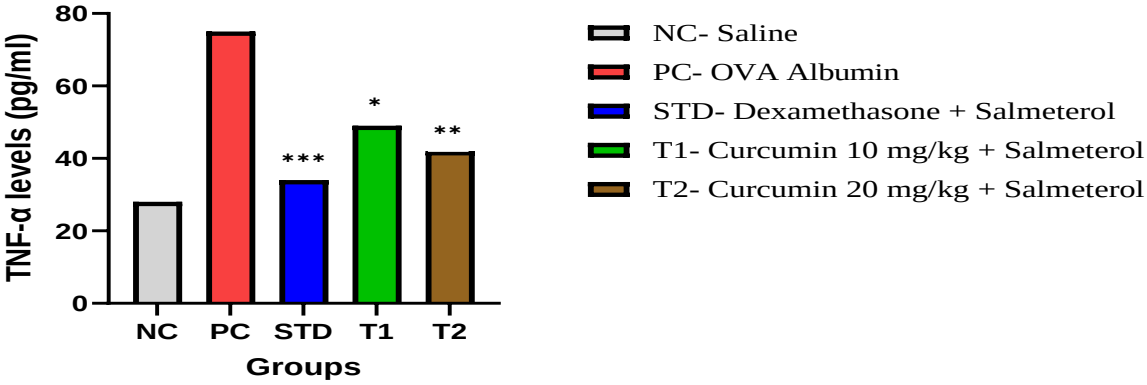


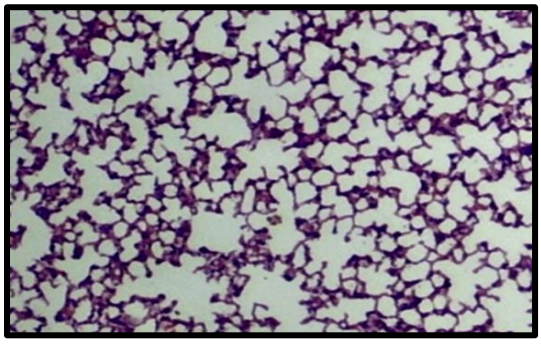
Fig. 10

Figs. 8,9,10. Represents -Impact of curcumin and salmeterol combination on IL-4, IL-13, TNF-α in lung tissue

*The result was evaluated using a one-way analysis of variance (ANOVA), following Dennett's Multiple Comparisons Test was then performed. The results are given as Mean ± S.E.M. (n = 6). A Positive Control Group was used to compare each group. non-significant, or ns-. **significant when compared to the Positive Control Group at P<0.05. NC stands for Normal Control. Positive Control (PC). STD: Standard group. T_1: treatment 1 median dose of curcumin T_2: Treatment 2 high dose of Curcumin*

3.3 Histopathological Examination

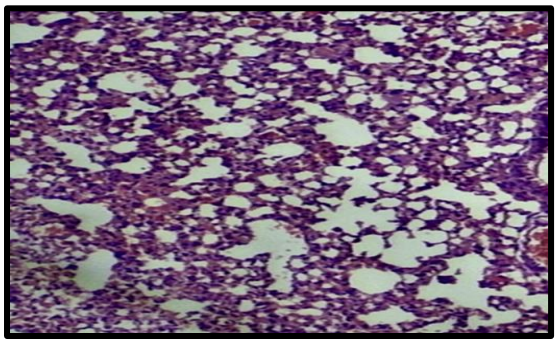
The lungs showed the pathological alterations mentioned as follows: Eosinophils and lymphocytes interstitial infiltration, infiltration grade, and further noteworthy abnormalities. There was no eosinophil or leukocyte infiltration, and no noticeable alterations within the lung tissues in the normal control group (Fig. 11). On the other hand, moderately to serious leukocytic infiltration was seen in the positive control group after mild eosinophilic infiltration (Fig. 12). There was also a small amount of hemorrhage visible. There was little leukocytic infiltration in the standard group (Fig. 13). Mild to moderate leukocytic infiltrations and little eosinophilic infiltrations were identified, same as in the treated groups. Nevertheless, treatment groups also exhibited minimal degree, similar to the positive control group (Figs. 14, 15).



NORMAL CONTROL

- No hemorrhage detected
- No eosinophilic/leukocytic infiltrations in the lungs were identified

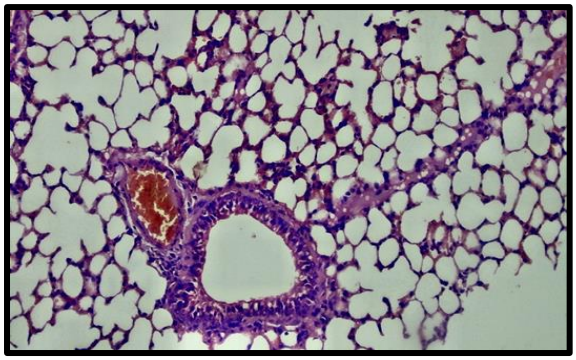
Fig. 11. Normal control



POSITIVE CONTROL

- Minimum degree of hemorrhage
- Mild to serious leukocyte invasion
- Mild eosinophilic infiltration

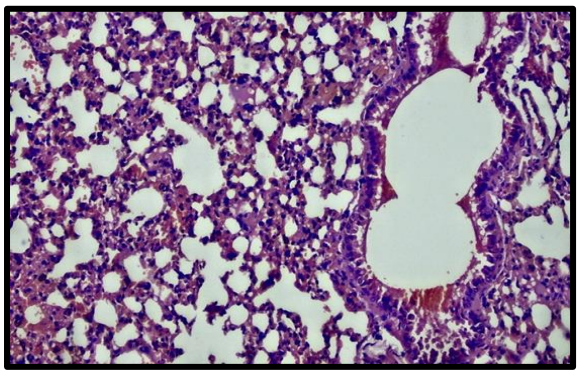
Fig. 12. Positive control



STANDARD GROUP

- No hemorrhage detected
- No eosinophilic infiltration
- limited infiltration of leukocytes

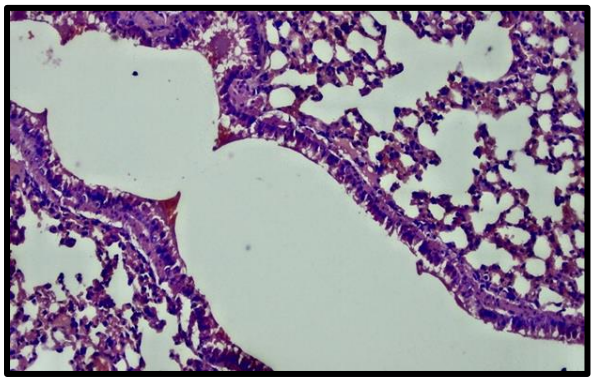
Fig. 13. Standard group



TREATMENT 1

- Minimal eosinophilic infiltration
- Mild to moderate leukocyte infiltration

Fig. 14. Treatment 1



TREATMENT 2

- No hemorrhage detected
- Minimal eosinophilic infiltration
- Mild leukocyte infiltration

Fig. 15. Treatment 2

Figs. 11-15. H&E and oil red O staining-induced alteration in the histopathology of tissue from lungs (11-15) Images illustrating the lung tissues of mice administered with (11) Normal Control (12) Positive Control, (13) standard group, (14) Treatment 1, (15) Treatment 2. Using 100X (H&E) magnification, pathophysiology analysis of the tissue portions was carried out via light microscopy

4. DISCUSSION

Asthma can be defined as persistent lung inflammation, an increase in eosinophils, mucus formation, Th2 cytokine excess production, elevated IgE levels, and hyperresponsive airways [24]. It has become a diverse condition as well as a worldwide epidemic that affects millions of humans and animals, prompting the rapid development of remedies to reduce its prevalence [25]. Multiple models of animals provide us with knowledge regarding the potential pathways associated with asthma etiology. The research project investigated the combined effect of curcumin and salmeterol in milk-induced leucocytosis and eosinophilia and OVA-induced asthma models. Curcumin in combination with Salmeterol reduced the amounts of eosinophils and leucocytes in milk-induced leucocytosis and eosinophilia. The same compound was administered to ovalbumin-challenged mice, which revealed elevated body weight, enhanced haematological parameters, blocked BALF cell infiltration, enhanced biochemical characteristics, reduced amounts of inflammatory cytokines, and enhanced lung tissue histology. Therefore, these findings demonstrated Curcumin's anti-asthmatic action in combination with Salmeterol using an OVA-challenged model of asthma and milk-induced leucocytosis and eosinophilia.

Leukocytes produce histamine, cytokines, and proteins that penetrate the tissue and enhance oxidative stress by producing oxygen species that are reactive, in addition to numerous other pathological characteristics of asthma. This is what causes asthmatic inflammation [26]. Cells associated with inflammation known as eosinophils are frequently observed within the submucosal and epithelium regions of bronchial biopsy collected from asthma sufferers. Eosinophilia is the term for an aberrant rise in peripheral eosinophil numbers that surpasses

4% of the overall leukocytes. The existence of eosinophils in the bronchial mucosa of asthma sufferers is linked with later asthmatic symptoms, such as excessive production of mucus and congestion. Eosinophils release mediators such as prostaglandin, eosinophil developed neurological poison, tumor necrosis factors, and eosinophil cationic protein during the later stages of hypersensitive asthma development. These cytokines cause inflammation, constriction of the bronchioles and epithelial sheds in the lungs, which is frequently allergic responses. Research has discovered that parenteral causes a notable and substantial rise in the leukocyte and eosinophil counts within twenty-four hours following the feeding. The group of mice which received a combination of curcumin and salmeterol treatment, according to the investigation outcomes showed a significant inhibition of rising overall leukocyte (Table 1 and Fig. 2) and eosinophil counts (Table 1 and Fig. 3) [27,28].

In an Ovalbumin-induced airways model, weakness in the muscles and lower 5-HT production in the brain caused the standard group of mice to weigh less than the ones in the positive control group. Additionally, dexamethasone increased plasma levels of leptin, indicating that leptin might contribute to anorexia brought on by dexamethasone. In contrast to the Positive Control group, the treatment combination groups body weights significantly improved (Table 2). One useful indicator of vascular permeability and edema is relative lung weight. In consequence of increased microvascular leakage, OVA-affected mice displayed greater lung edema; however, treatment combination groups demonstrated a significant reduction in relative lung weight (Table 2) [29].

Asthma and other inflammation related conditions depend heavily on the regulation of the immune system by white blood cells (WBC), which include neutrophils, eosinophils, lymphocytes, and macrophages. The presence of neutrophils among individuals with asthma or allergic responses makes WBC recognition medically pertinent [30]. The total white blood cell count (WBC) increased significantly in the investigated positive control groups. Moreover, there was a rise in the differential cell population, which includes neutrophils, lymphocytes, macrophages, and eosinophils. The results of this research demonstrated a significant reduction in the total and differential cell counts with the use of the therapy combination of salmeterol and curcumin (Table 3). One of the main characteristics of allergic asthma is inflammation, which raises eosinophil counts and causes increased responsiveness in the bronchi and inflammatory responses that destroy the extracellular matrix and cells of the endothelial in the airways. The introduction of the combination of treatments suppressed the considerable abnormalities observed in the total and differential cell count found in the BAL fluid which was increased in case of positive control group in comparison to the normal control group (Table 4). One of the main characteristics of inflammation of the airway is the tearing of epithelial cells. The current research shows that the positive control group had higher levels of airway epithelial cell tearing, an indication of airway inflammation, which was successfully inhibited by the treatment combination (Table 4) [10]. Mast cells, eosinophils, and basophils are then made more sensitive to allergic substances by IgE antibodies attaching to their Fc epsilon receptor I (FcεRI) receptor [31]. IgE is a major stimulator of Th2 cell release in the pathophysiology of asthma, and T cells are the main agents of allergic disorders [16]. According to our research, the combination treatment drastically reduced the overall IgE levels in serum, while the positive control groups levels of IgE in serum raised (Fig. 4).

An important factor in the development of asthma is inflammation [30]. Th 2 cytokines such as IL-4 and IL-13, which are released by lungs living cells such as bronchial epithelial cells, tissue mast cells, alveolar macrophages, and inflammatory cells including lymphocytes and eosinophils, are important in allergic inflammation of the airways [32]. Although IL-13 directly promotes excessive secretion of mucus and hyper responsiveness in the airway while IL-4 is essential for Th2 inflammatory reaction, IgE production, and B cell development [33]. Th2 cells signals, primarily IL-4, which are needed for B lymphocytes to switch to produce IgE and for macrophages and dendritic cells to become activated. AHR occurs in the hypersensitive lungs when IL-13 uses IL-4-mediated signal transduction to stimulate the transcription factor and signal transducer STAT 6. AHR is the clinical manifestation of asthma. Cytotoxic granule proteins released by infiltrating inflammatory cells into the lungs may result in lung damage [34]. As a result Th2 cytokines are important in controlling allergic reactions. Within the positive control

group, there was a substantial spike in the levels of IL-4 and IL-13 in both the lung tissue and BALF. The treatment of Curcumin and Salmeterol decreased the number of cytokines associated with Th2, IL-4 and IL-13, in both lungs tissue (Figs. 8,9) and BALF (Figs. 5,6) based on the findings of the study.

Asthma-related inflammation is mediated by TNF-α, a cytokine that promotes inflammation secreted by NK cells and monocytes [35]. It enhances the attachment of inflammatory cells (such as neutrophils and eosinophils) to the airway surface, activates the smooth muscle of the airways, and raises the expression of adhesion molecules. Additionally, it triggers the production of cell adhesion molecules, the expulsion of inflammatory mediators by eosinophils, and the activation of myofibroblasts [24]. High levels of TNF-α in BAL fluid in asthmatics cause contractions of the airways. In light of these findings, TNF-α levels in lung tissues and BALF were examined. Following OVA challenge, there was an evident elevation of TNF-α concentrations among positive control animals. On the other hand, treatment with curcumin and salmeterol markedly decreased TNF-α concentrations in lung tissues (Fig. 10) and BALF (Fig. 7), suggesting that it has anti-inflammatory properties against immunoinflammatory disorders caused by allergens.

Elevations in albumin and protein concentrations are believed to be a sign of tissue injury [36]. In OVA-sensitized mice, the investigation looked at the effects of several treatment combinations on total protein (TP) and albumin concentrations. It was discovered that curcumin and salmeterol inhibited a rise in TP levels. In contrast to the normal control group, a notable rise in the TP levels was noted in the lung tissues, serum, and BALF of the positive control group (Table 5).

Curcumin and Salmeterol could revolutionize asthma treatment by providing a comprehensive approach to managing inflammation and airway constriction. This combination may reduce the need for high doses of corticosteroids and improve overall asthma control. However, clinical trials are needed to determine safety, efficacy, and optimal delivery methods.

5. CONCLUSION

Asthma drug therapies now on the market are accompanied with multiple negative consequences and are aimed at managing asthmatic worsening symptoms. According to the current research findings, curcumin and salmeterol together have anti-asthmatic properties against allergic asthma produced by OVA and leucocytosis and eosinophilia induced by milk in mice. The anti-asthmatic and anti-allergic properties of the therapy combination were demonstrated by the reduction in eosinophil and leucocyte levels. The anti-inflammatory effect of the treatment combination was demonstrated via a reduction in the blood's and BALF's overall and differential cell numbers. The combination of treatment additionally displayed immune-modulating properties, as seen by improvements in serum IgE levels, overall protein content, the amount of albumin, cytokines associated with Th2 (IL-4 and IL-13), immune-inflammatory response (TNF-α) in lung tissues, as well as BALF. These findings imply that the combined benefits of salmeterol and curcumin could potentially be utilized as a "add-on therapy" for individuals with asthma, or they may be used in addition with the existing anti-asthmatic medications now on the market.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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