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Effects of COX Inhibitors on Responsiveness of the Tracheal Tract to Acetylcholine and Histamine and their Relationship with LTC₄ and PGE₂ Levels of Bronchoalveolar Lavage Fluid in Allergic Guinea Pigs

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Abstract:

Introduction: Nonsteroidal anti-inflammatory drugs (NSAIDs) intervene in the COX (cyclooxygenase) pathways which generate two important inflammation mediators, prostaglandins (PGs) and leukotriene (LTs). Contradictory claims regarding the effect of NSAIDs in asthmatic patients continues to be an issue. The present study investigated the effects of COX inhibitors on the responsiveness of the tracheal tract and on the levels of LTC₄ and PGE₂ in cells of the bronchoalveolar lavage fluid in an allergic guinea pig model.

Materials and Methods: Adult male Dunkin-Hartley guinea pigs (250 - 300 g) were divided into seven groups of six animals each. Four COX inhibitors, aspirin (200 mg/kg and 20 mg/kg), indomethacin (10 mg/kg), ketoprofen (10 mg/kg), and celecoxib (25 mg/kg), were given orally on day 17 to allergy induced guinea pigs at 0, 12, and 24 h before ovalbumin challenge on day 18. PGE₂ and LT₄ were measured in the bronchoalveolar lavage fluid as well as inflammatory cell count and total protein. Tracheal responsiveness to acetylcholine (Ach) and histamine (His) also was evaluated. **Results:** An augment in the response of the trachea to Ach and His, as well as overt allergenic signs including short breath, wheezing and sneezing, was observed. The most significant increase in tracheal hyper-responsiveness was observed in the ketoprofen-treated group with similar but less pronounced changes observed in the indomethacin-treated group. Although some variables increased with the aspirin and celecoxib treatments, overall the tracheal sensitivity was reduced. Inflammatory cells including eosinophils and neutrophils corresponded to the changes observed for each treatment group. **Conclusion:** Ketoprofen and indomethacin increased the tracheal sensibility to Ach and His; therefore, their administration is not recommended in patients susceptible to allergy.

Keywords: Allergy, Hyper-responsiveness, NSAIDS, COX Inhibitors, Trachea

Introduction:

Asthma is a chronic respiratory disease associated with bronchial and pulmonary inflammation and its main signs include reversible airflow obstruction and bronchospasm. Airway hyper-responsiveness is a characteristic of bronchial asthma (1). Human-like asthma disorders can be modeled in several animal species including dogs, cats, guinea pigs, and horses(2)

Aspirin-like or nonsteroidal anti-inflammatory drugs (NSAIDs) are a class of drugs used for the treatment of inflammatory disorders (3, 4). Previous studies have shown that aspirin-like drugs can lead to bronchial constriction and increased airway responsiveness to bronchial spasmogenic compounds in some asthmatic patients (Aspirin-induced Asthma; AIA) (5), which if not treated can result in mortality (6).

Prostaglandins and leukotrienes are eicosanoid like chemicals (derivatives of 20-carbon polyunsaturated fatty acids) which can mediate inflammatory reactions including asthma. These chemicals can increase the severity of asthma in patients. Cyclooxygenase (COX) is the key enzyme that catalyzes prostaglandin synthesis from arachidonic acid (derived from plasma membrane phospholipids). This enzyme has two forms: 1) COX-1 which is continuously expressed in most cells and 2) COX-2 that is an inducible form of the enzyme that is produced in cells by stimulation of the tumor necrosis factor (TNF- α) and interleukin-1 as well as certain growth factors including epidermal growth factor (EGF) (7). Products of COX or prostaglandins (PGs) are key regulators of allergic inflammation of the respiratory tract, including asthma. Such products can potentially alter airway tone and can trigger an inflammatory response (8).

The mechanism(s) involved in AIA (Aspirin-Induced Asthma) is not understood yet. However, it has been hypothesized that reduction of arachidonic acid conversion into prostaglandins can lead to greater arachidonic acid levels, making the acid available for leukotriene production via the lipoxygenase pathway (9). On the other hand, inhibition of PGE₂ synthesis has been suggested to be involved in AIA development since PGE₂ is a known bronchoprotective agent that induces bronchodilation (10).

Due to their widespread availability, NSAIDs are used to reduce Aspirin-induced Asthma symptoms (11). However, it has been reported that some categories of NSAIDs can trigger the AIA response in patients (12). So far, however, no reports have compared the *in vivo* effects of such enzyme inhibitors involved in prostaglandins synthesis on airway responses or have assessed different aspirin-like NSAIDs on airway responsiveness. Since there are conflicting data currently available, this study was designed to evaluate the effects of this class of drugs on airway responsiveness as well as to investigate the effects of celecoxib (preferential COX-2 inhibitor) on the airways of guinea pigs.

Materials and Methods:

Animals

Forty-two adult male Dunkin-Hartley guinea pigs (250-300 g) were purchased from the Pasteur Institute, Iran. The animals were individually housed under controlled environmental conditions at 19 – 25 °C with a relative humidity of 50 – 60% and a 12 h light/dark cycle with free access to feed pellets and drinking water at the Faculty of Veterinary Medicine, University of Tehran. In order to provide the vitamin C requirement, a vitamin C powder (Osveh Pharmaceutical Company, Iran) was dissolved daily in their drinking water (1g/L). The care and use of laboratory animals was in accordance with the guidelines established by the Iranian National Institute of Health and the protocol for this study was approved by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Tehran (approval No of 7508134.3.11). After seven days, the animals were randomly divided into seven groups of six animals each. The dose selection for this study was based on literature values (13-15).

1. Control group (allergy not induced)
2. Allergic group (allergy induced without NSAIDs treatment)
3. Allergic group treated with high dose of aspirin (200 mg/kg)
4. Allergic group treated with low dose of aspirin (20 mg/kg)
5. Allergic group treated with indomethacin (10 mg/kg)
6. Allergic group treated with celecoxib (25 mg/kg)
7. Allergic group treated with ketoprofen (10 mg/kg)

Treatment

In all the treatment groups (see above), allergy was induced using the protocol of de Boer et al by i.p. administration of a 2 mL aqueous suspension containing ovalbumin (150 µg/animal) and aluminum hydroxide (150 mg/animal) on days 1, 5, and 8 (16). On day 17, guinea pigs in all groups (except groups 1 and 2) received the respective drug orally at the following time points, 0, 12, and 24 h before being challenged. On day 18, animals were challenged with a 0.5% solution of ovalbumin by inhalation for a maximum period of 5 min. Animals in the control group received normal saline (16). All the experiments were approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, University of Tehran, Iran.

Bronchoalveolar Lavage Fluid (BALD) Extraction and Tissue Preparation:

On day 19, twenty-four h after being challenged with ovalbumin, guinea pigs were euthanized with an i.p injection of thiopental sodium (100 mg/kg) (17). Immediately after death, the trachea was exposed and cut from the larynx by a longitudinal incision on the neck. Five mL of Krebs' solution was infused three times into the lungs using an endotracheal tube at 37 °C and after one min the liquid was collected and centrifuged. The supernatant fraction was used for total protein and the remained liquid was kept at -70 °C for PGE2 and LTC4 measurements. The sediment after centrifugation was used for evaluation of inflammatory cells. The trachea from each guinea pig was cut into three sections each consisting of four cartilage rings. These sections were placed in an organ bath with warm water.

Evaluation of Trachea Responsiveness to Acetylcholine and Histamine

The response of the tracheal sections to acetylcholine and histamine was measured in a 4-channels organ bath (LSI Lecta, Spain) connected to a Power Lab (AD Instruments, Australia) (18). Dose-response curves were obtained based on the cumulative concentrations of acetylcholine and histamine. The resulting curves were used to estimate maximum responses (E_{max}), effective concentrations (EC_{25} , EC_{50} and EC_{75}) at 25, 50, and 75% and for comparing the concentration gradients among groups.

Measuring PGE2, LTC4, and Total Protein:

The supernatant fraction following centrifugation at 1000 r.p.m. for 7 min (19) of a bronchoalveolar lavage fluid (BALF) was evaluated for PGE2 and LTC4 using an ELISA method according to the company's instructions (Cayman Chemical Company, MI, USA). Total protein was measured by the Lowry method (20).

Statistical analysis:

Data are expressed as means $\pm$ SEM. Statistical significance was analyzed with a one-way analysis of variance (ANOVA), followed by the Bonferroni test as a post hoc analysis for individual comparisons. Differences with $P < 0.05$ were considered statistically significant.

Results:

Overt allergenic signs including short breath, wheezing, and sneezing were observed in all the allergenic guinea pigs. A comparison of changes in the number of inflammatory cells between the control group (normal lung tissue) and the allergic group is present in Figures 1 and 2. Severe invasion of inflammatory cells (mostly eosinophils) occurs in the alveolus and tissue of the allergic guinea pigs but not in control animals.

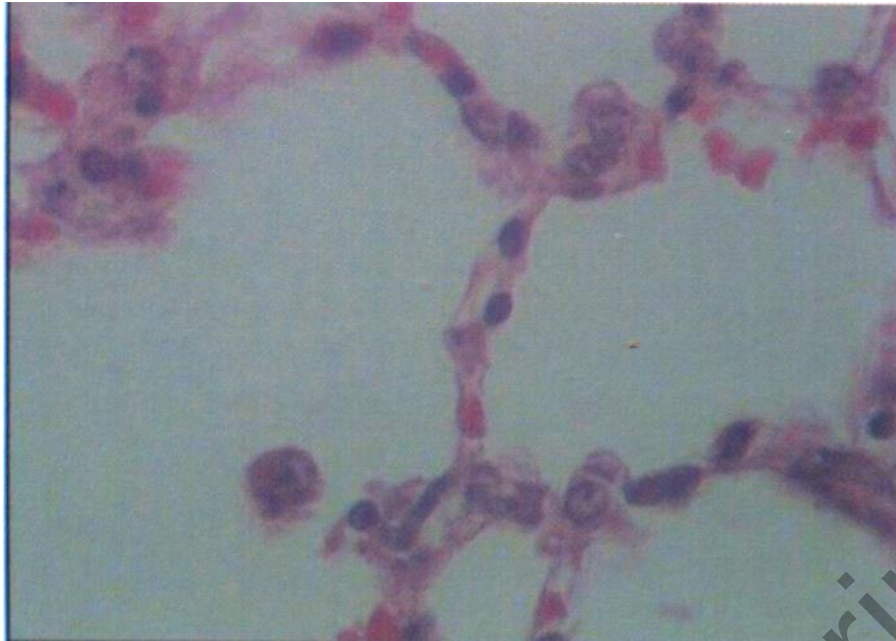


Figure 1: Alveolar tissue in control guinea pigs. H&E: $\times 400$. H&E: hematoxylin and eosin

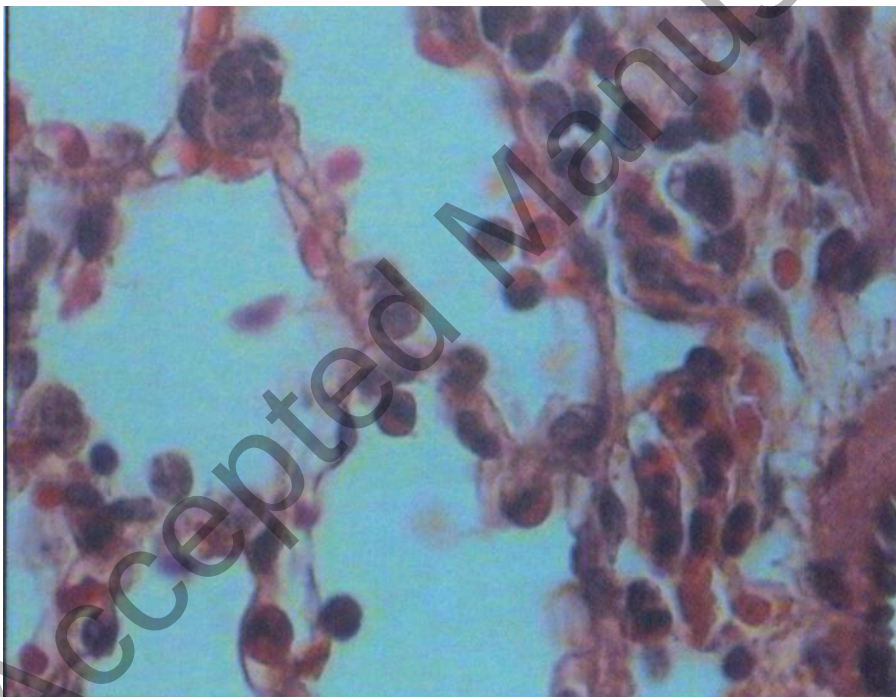


Figure 2: Lung tissue from a guinea pig in the allergic group. Severe invasion of inflammatory cells (mostly eosinophils) occurs in alveolar tissue. . H&E: $\times 400$. H&E: hematoxylin and eosin

Tracheal Response to Acetylcholine

$E_{\max}$

The $E_{\max}$ is a scale to measure the maximum contraction response or ultimate traction to a transducer (gr). This response gives an indication of the amount of pressure or tension in comparison with normal tension of control tracheal tissue. The maximum contractile responses to indomethacin and ketoprofen were 4.2 and 5.3 times greater, respectively, than the response seen in control guinea pigs ($p < 0.001$) (Fig 3). In addition, these increases were statistically significant compared to the allergic group (Group 2) and the other treatment groups ($p < 0.01$).

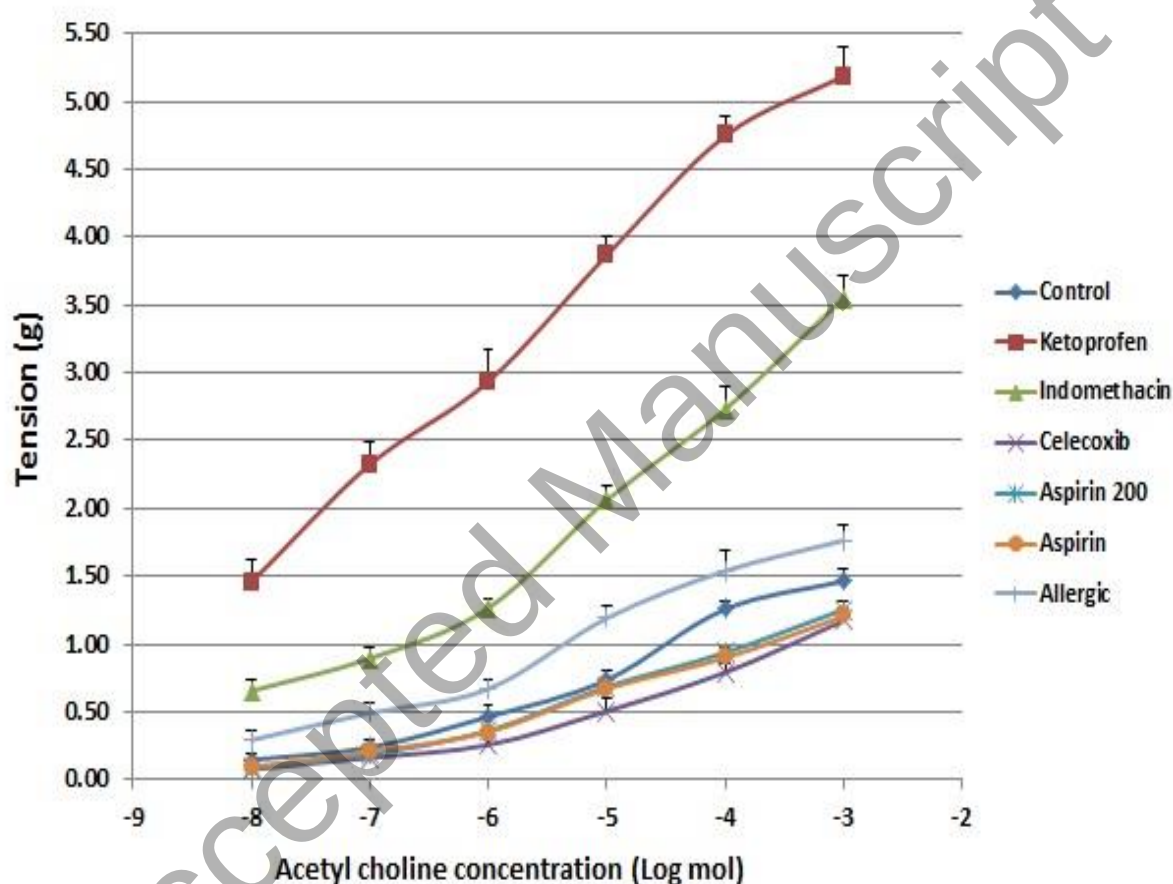


Figure 3. Acetylcholine dose-response. The values show the tracheal response to various treatments in the presence of acetylcholine. Data are mean $\pm$ SEM.

E_{25} , E_{50} and E_{75} :

The $E_{25\%}$, $E_{50\%}$ and $E_{75\%}$ response values for the indomethacin, ketoprofen, and the allergic groups were increased compared to the other groups. The $E_{25\%}$ value for celecoxib and both aspirin doses showed significant reductions compared to the other groups, including animals in the control groups.

Tracheal Responses to Histamine:

$E_{\max}$

The maximum contractile responses in the indomethacin and ketoprofen groups were 2.2 and 2.5 times greater than that of the control group, respectively ($p < 0.001$). In addition, these increases were statistically significant compared to the allergic and the other treatment groups ($p < 0.01$) (Fig 4)

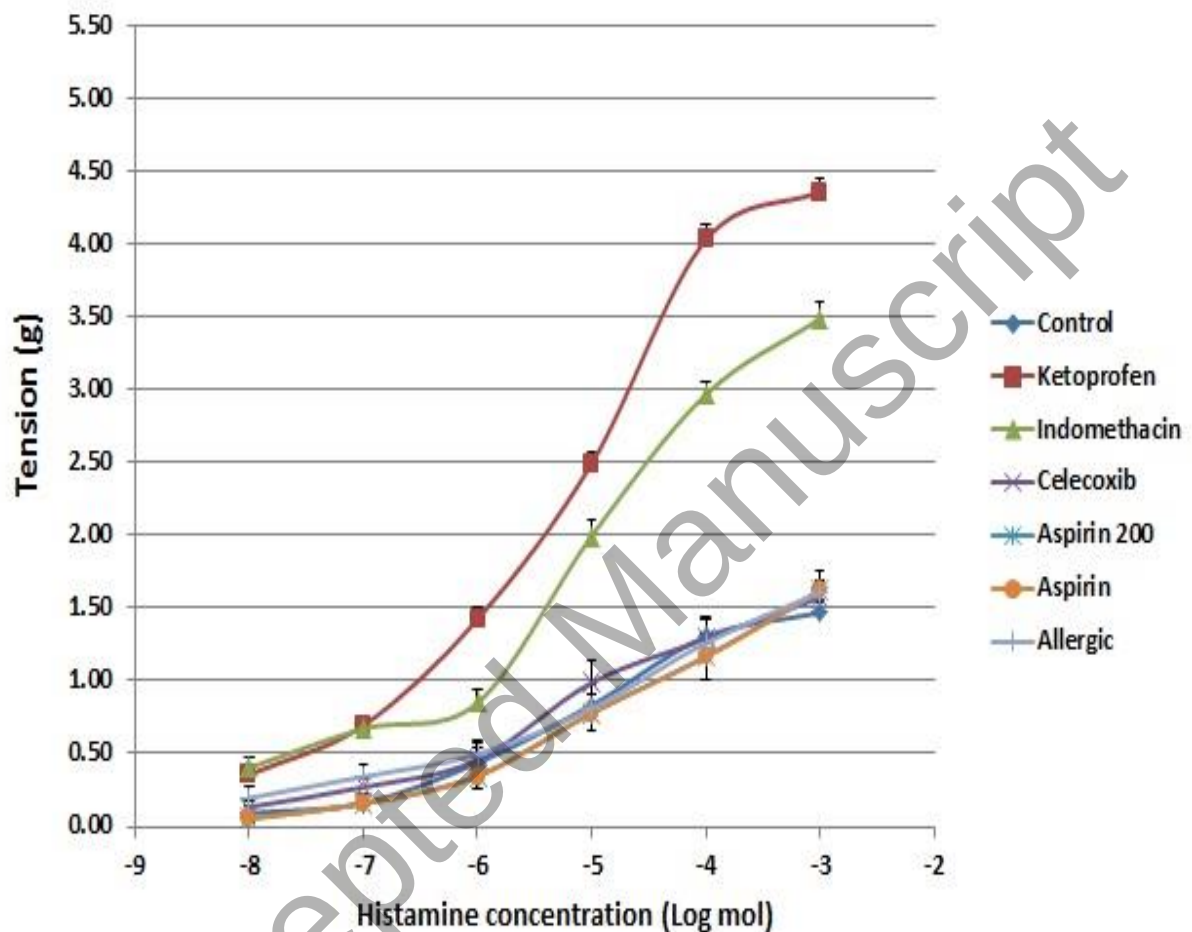


Figure 4. The values show the tracheal response to various treatments in the presence of histamine. Data are shown as mean $\pm$ SEM. ($E_{\max} = 10^{-3}$).

E_{25} , E_{50} and E_{75} :

As was the case for acetylcholine treatment, the $E_{25\%}$, $E_{50\%}$ and $E_{75\%}$ values for the indomethacin and ketoprofen groups were increased compared to the other experimental groups and the control group, except for the allergic group (Group 2). The $E_{25\%}$ values for celecoxib and aspirin (both doses) showed a significant reduction compared to the allergic group.

PGE2 levels:

Although induction of allergy led to increased levels of PGE2 compared to the control group, decreased levels of PGE2 in the ketoprofen and both aspirin groups were observed, while increases occurred in the indomethacin and celecoxib groups (Group 5). However, none of these values were statistically significant compared to allergic group. The indomethacin and celecoxib treated guinea pigs had significantly increased PGE2 levels compared to the ketoprofen treated animals and to both of the aspirin-treated groups.

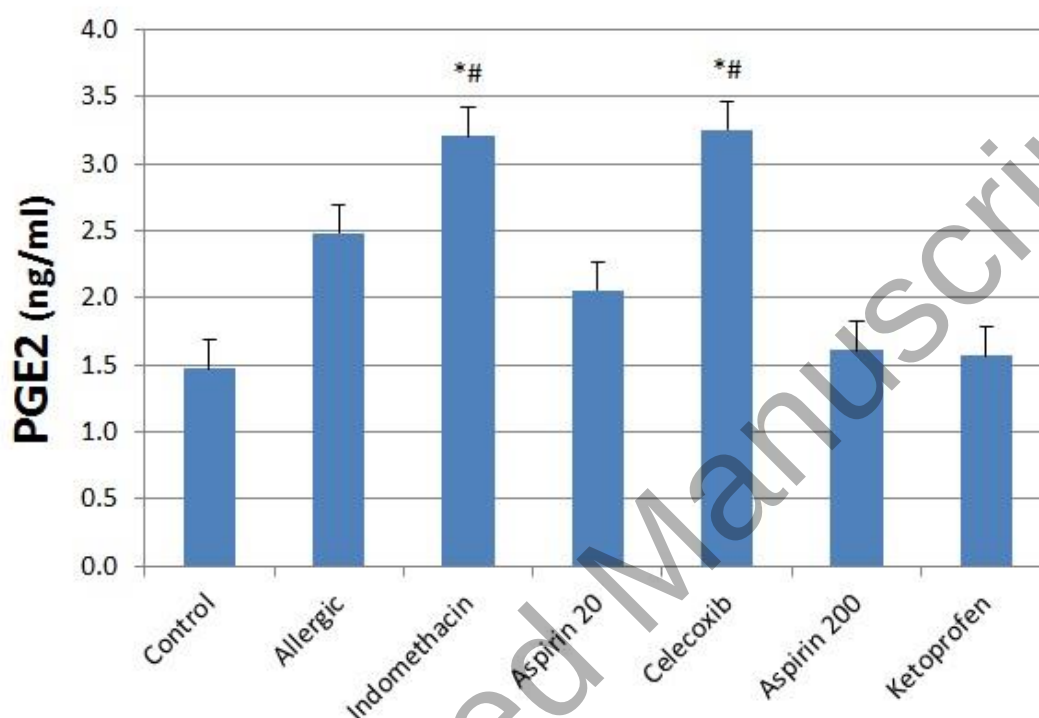


Fig 5: The values of PGE2 (ng/mL) were obtained from BALF (Bronchoalveolar Lavage Fluid). Data are shown as mean $\pm$ SEM.

Significant difference between mentioned groups and control group ($P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

#Significant difference between mentioned groups and allergic group (# $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$).

LTC4 levels:

Similar to PGE2, induction of allergy in the guinea pig model induced a significant increase in LTC4 levels compared to control animals ($p > 0.05$) (Figure 4). Compared to the allergic group, a decline was observed in the BALF LTC4 levels in the indomethacin, ketoprofen, and aspirin (both doses) groups, with a moderate increase in the LTC4 level in celecoxib treated guinea pigs. The indomethacin and the aspirin 200 mg/kg doses were statistically significantly different from the allergic guinea pigs.

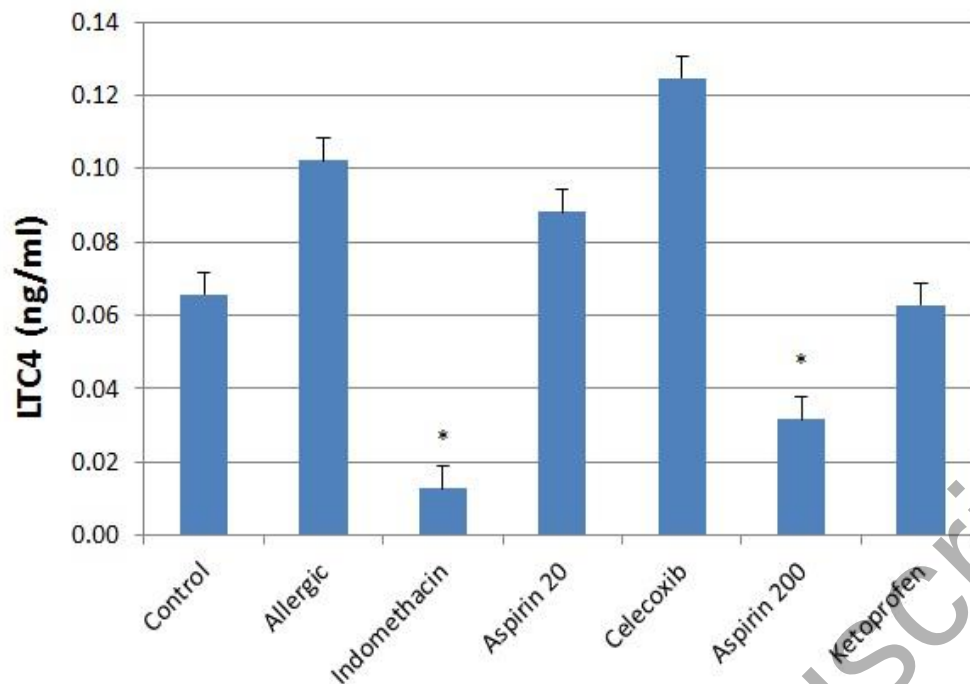


Fig 6: LTC₄ levels (ng/mL) following exposure to various drugs in the guinea pig allergenic model. Induction of allergy in the guinea pig model caused a significant increase in LTC₄ levels compared to control animals ($p > 0.05$). Compared to the allergic group, a decline was observed in the BALF LTC₄ levels in the indomethacin, ketoprofen, and aspirin (both doses) groups, with a moderate but not significant increase in the LTC₄ level in celecoxib treated guinea pigs. The indomethacin and the 200 mg/kg doses were statistically significant. Values are mean $\pm$ SEM.

* Significant difference between mentioned groups and control group (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Significant difference between mentioned groups and allergic group (# $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$).

Total white blood cells:

The total number of white blood cells in the BALF of allergic and all the treated groups increased significantly compared to that of control animals (Fig 7). This parameter in the low-dose aspirin and celecoxib groups was significantly lower than that of the allergic group.

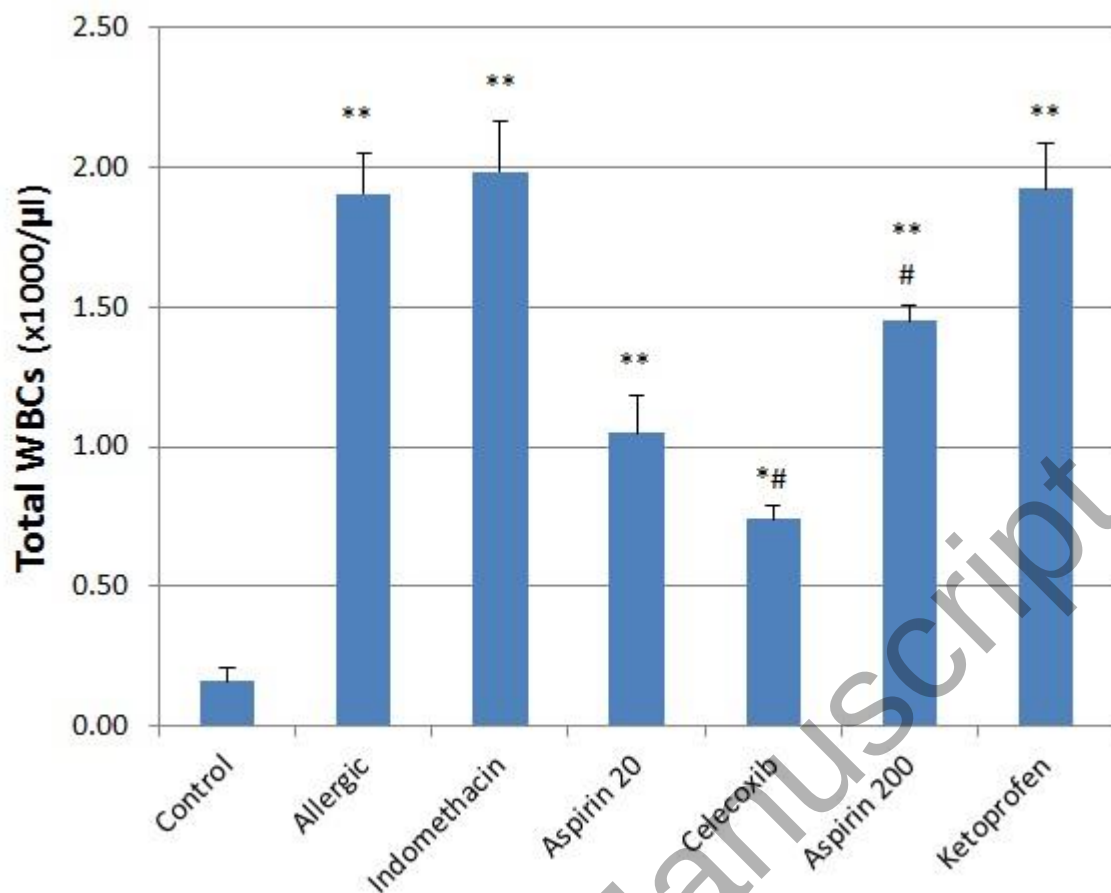


Fig 7: The total number of white blood cells (x 1000/uL) in the BALF of allergic** and all treated groups*** increased significantly compared to the WBCs in the control animals. This parameter in low-dose aspirin and celecoxib groups was significantly lower than that of the allergic group. Values are mean $\pm$ SEM

* Significant difference between mentioned groups and control group (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Significant difference between mentioned groups and allergic group (# $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$).

Eosinophils, Lymphocytes, and Neutrophils:

Although all treatment groups had elevated levels of WBC in BALF when compared to the control group, the percentages of lymphocytes were decreased in all experimental groups in comparison with the control animals (Fig 10). There was a non-significant increase in the neutrophil count in the allergic group. Guinea pigs treated with ketoprofen, indomethacin, high-dose aspirin, and celecoxib had an increased percentage of neutrophils compared to the control group ($p < 0.05$) (Fig 9).

The average percentages of eosinophils in the control and allergic groups were 2.8 ± 0.7 and 51.1 ± 3.6 , respectively. The increase of eosinophil in the allergic group was statistically significant compared to the control group. All of the treatment groups had an increased percentage of eosinophils compared to the control group ($p < 0.001$) (Fig 8).

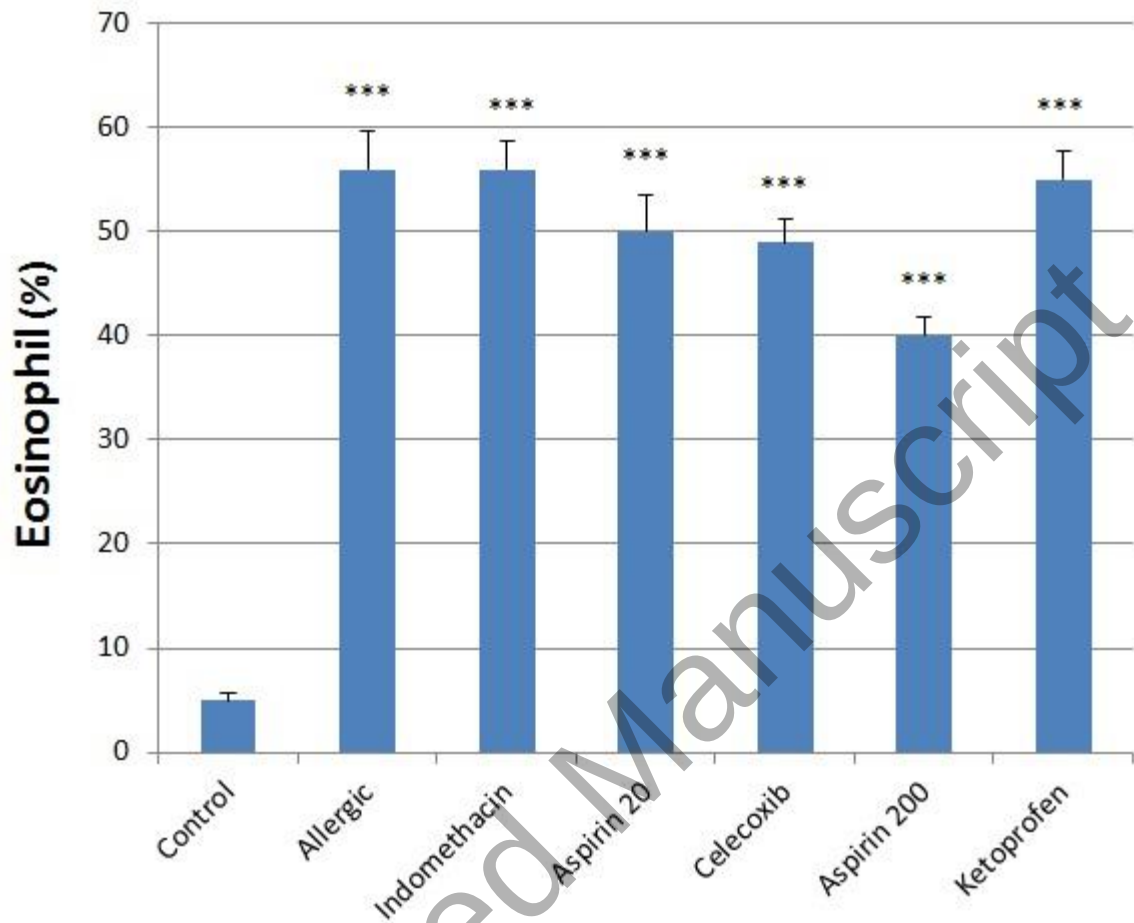


Fig 8: The values of eosinophil obtained from BALF (Bronchoalveolar Lavage Fluid) 24 h after challenging. Data are shown as mean $\pm$ SEM.

* Significant difference between mentioned groups and control group (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Significant difference between mentioned groups and allergic group (# $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$).

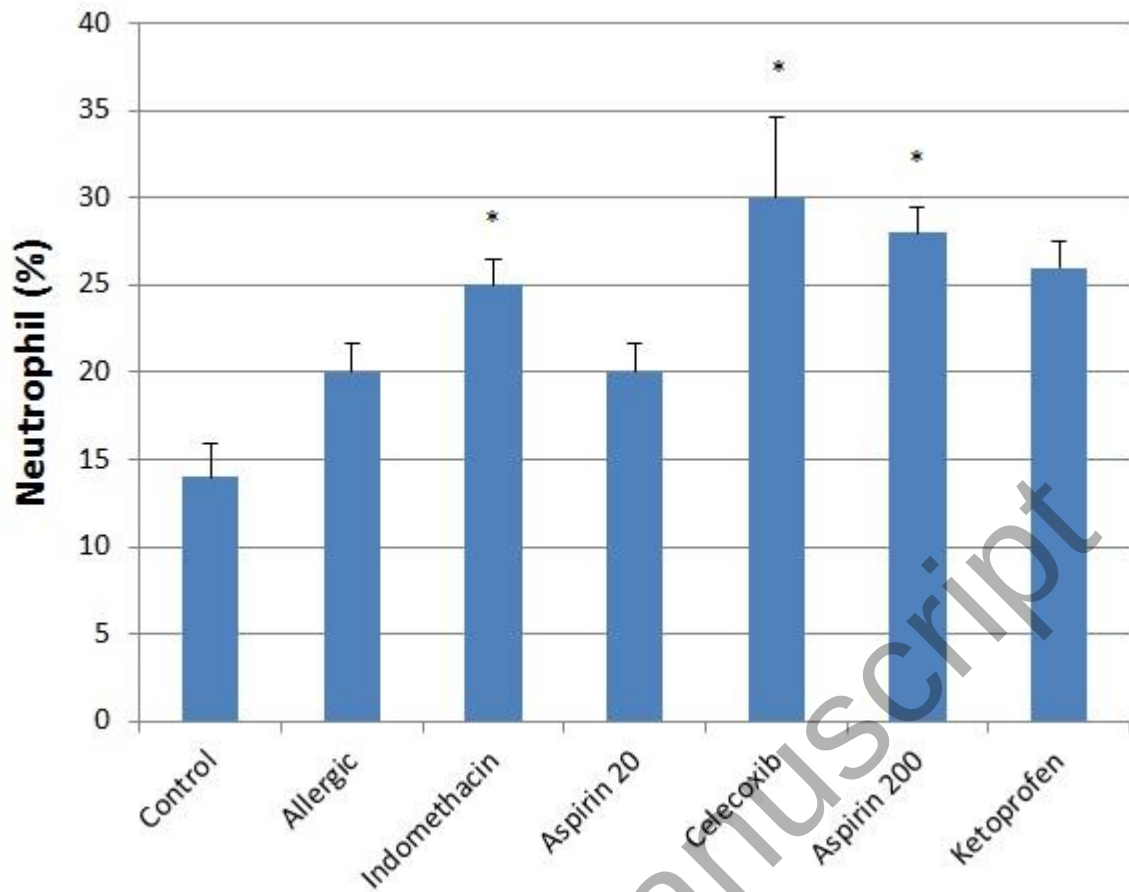


Fig 9: The values of neutrophil obtained from BALF (Bronchoalveolar Lavage Fluid) 24 h after challenging. Data are shown as mean $\pm$ SEM.

* Significant difference between mentioned groups and control group (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Significant difference between mentioned groups and allergic group (# $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$).

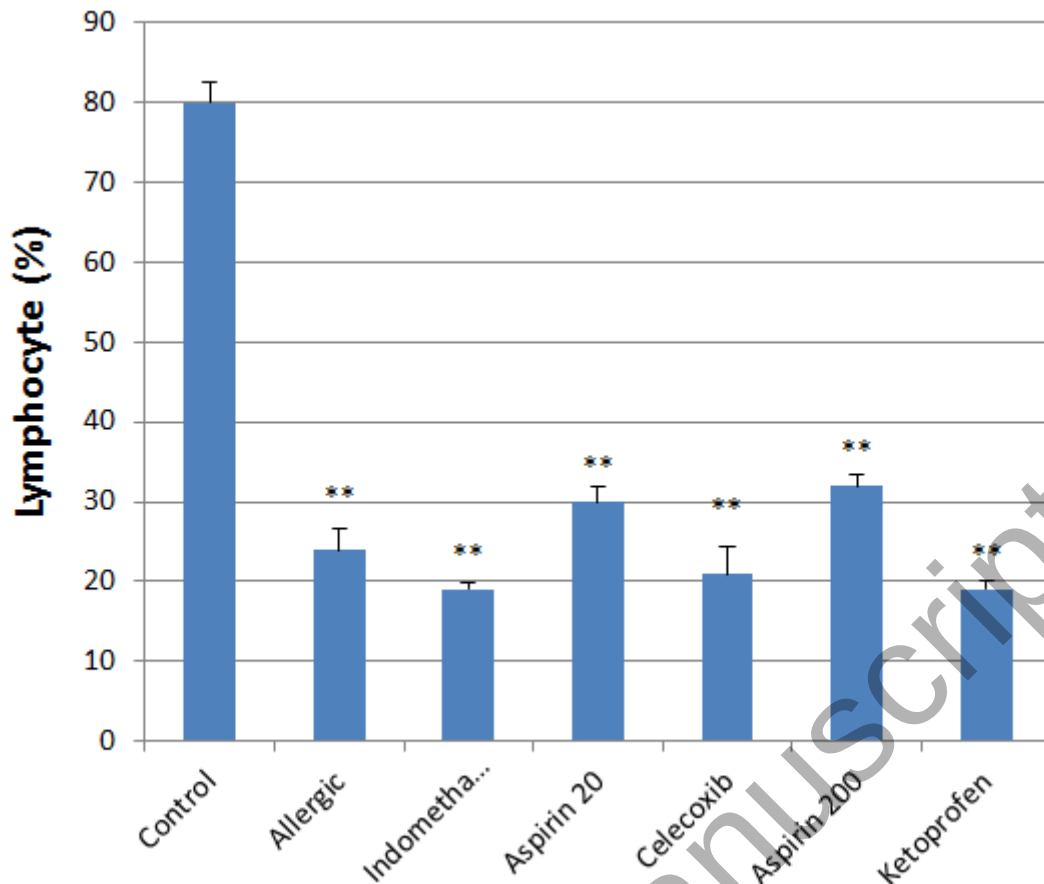


Fig 10: The values of lymphocyte obtained from BALF (Bronchoalveolar Lavage Fluid) 24 h after challenging. Data are shown as mean $\pm$ SEM.

* Significant difference between mentioned groups and control group (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Significant difference between mentioned groups and allergic group (# $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$).

BALF Total protein levels:

No significant differences were observed in the BALF total protein levels among the experimental groups 24 h post-challenge with ovalbumin.

Discussion:

This study investigated the effects of four COX inhibitors on tracheal responsiveness and their relationship with BALF PGE₂ and LTC₄ levels in an allergic guinea pig model. PGs and LTs are two key regulators of allergic inflammation of the respiratory tract that can alter the inflammatory response and airway tone (21). These inhibitors have various effects on the inflammation process but are thought to have a pathogenic role in asthma. PGs and LTs attract neutrophils to inflammation sites, increasing proliferation of bronchial smooth muscles and participate in the inflammatory response (22). Inhalation of cys-LTs can also result in increased tracheal response to histamine (23). In Szczeklik et al. (1999) research, their results demonstrated that aspirin can augment eosinophilic inflammation and overproduction of leukotrienes (24).

Airway hyper-responsiveness (AHR) is one of the important signs of allergic asthma and can result from pulmonary inflammation and subsequent release of prostaglandins and leukotrienes (25). COX inhibition by COX-2 selective or non-selective drugs can lead to AHR in allergic animal models in addition to increasing inflammation (26). The inhibition of the COX enzyme in an allergic mouse model lacking active lipoxygenase (the enzyme which catalyzes the synthesis of leukotrienes) increased airway responsiveness (27). In addition, it was reported that the lack of COX-1 increased AHR and the production of leukotrienes (28). In an earlier study, it was reported that aspirin-like drugs such as indomethacin increased *in vivo* airway responsiveness in guinea pig (13). Babu, K et al. (2000) showed that AIA decreased PGE₂, but that it increased cysteinyl leukotrienes. The suggested pathogenesis is related to aspirin-like drugs shifting from the COX pathway to lipoxygenase pathway (29).

In the present study with male guinea pigs, as well as studies in mice and guinea pigs (30), challenge with ovalbumin increased tracheal responsiveness to acetylcholine and histamine in an isolated organ bath system. After challenge with ovalbumin, signs such as shortness of breath, increased respiratory rate, wheezing and sneezing were observed in treated animals. Oral administration of indomethacin and ketoprofen (10 mg/kg) significantly increased tracheal responsiveness to all concentrations of histamine and acetylcholine after ovalbumin challenge in a dose-response manner compared to the allergic group guinea pigs. Indomethacin and ketoprofen caused a shift of the dose response curve and increased isolated tracheal sensitivity to small amounts of histamine and acetylcholine.

The *in vitro* response of the trachea and bronchial smooth muscle in monkeys, dogs, and guinea pigs to histamine and acetylcholine has been reported to increase significantly in the presence of indomethacin and ketoprofen (14, 31). Similar results were obtained with human trachea. Moreover, tracheal and bronchial smooth muscle in the presence of indomethacin and some other aspirin-like drugs showed a similar contractive response to histamine and acetylcholine. It has been demonstrated that COX inhibition by indomethacin significantly increased the tracheal response to several allergen (15).

Few studies have investigated the effects of ketoprofen in aspirin-induced asthma. However, in most studies including the present study, ketoprofen improved the exacerbation of symptoms in asthmatic patients (32). In another study conducted on guinea pigs, administration of lysine-ketoprofen (100 µg/kg) reduced leukotriene C₄ and bradykinin-induced bronchial hyper-responsiveness two h after exposure, probably due to inhibition of prostanoids. A number of case reports in humans have indicated the risk of an asthma attack after taking ketoprofen or even using ketoprofen adhesive agents (33). Thus far to the best of our knowledge, no studies have investigated the ketoprofen effects on the airways of laboratory animals with induced allergy.

Lymphocytes were decreased in all the experimental groups compared to the control group in our study using a guinea pig allergenic model. The decrease in the lymphocyte count occurred because the NSAIDs can inhibit lymphocyte infiltration and migration in inflamed tissues. Paskauskas et al. (2015) have speculated that NSAIDs can enhance inhibition of calcium metabolism (34) or intracellular ATP (35) to inhibit leukocyte migration, thus decreasing the total number of lymphocytes (36). In contrast, an increase in lymphocyte counts was shown in aspirin-induced asthma due to a viral infection which may be due to invasion of lymphocyte as a main cell defense to infected cells (37).

Cys-LTs are involved in accumulation of eosinophils in asthmatic airways (38). In an article by Ogata et al (1999), it was reported that this LT family plays an important role in eosinophilic lung inflammation in allergic mice treated with indomethacin (39). An alternative hypothesis suggests that indomethacin or COX inhibition can increase the level of IL-5, which in turn, results in augmentation in eosinophils accumulation, growth, and survival. Similar to previous studies, our results showed that both indomethacin and ketoprofen exacerbated signs of respiratory allergies in ovalbumin-sensitized guinea pigs. Although ketoprofen is a non-selective COX inhibitor, it can be concluded that ketoprofen increased the allergic reaction signs through a mechanism similar to indomethacin (40).

In previous studies it was reported that usage of COX-2 inhibitors did not increase the allergic response in histamine-allergic mice. Conversely, these inhibitors significantly decreased the infiltration of eosinophils and neutrophils in the bronchoalveolar lavage. In the present study, celecoxib did not increase tracheal sensitivity to the allergic response but instead, reduced the tracheal reaction to acetylcholine and histamine compared to the allergic group. Administration of aspirin (20 mg/kg) resulted in a similar outcome to celecoxib treatment. It is hypothesized that less eosinophils and inflammatory cells in the alveolar tissue lead to reduced responsiveness of the trachea to the allergic response.

In general, selective COX-2 inhibitors such as celecoxib are well tolerated in most patients with AIA. Selective COX-2 inhibitors like celecoxib may be suitable alternatives for AIA patients who cannot tolerate non-selective COX inhibitors (41, 42). Celecoxib has been used to sort out potential differences in PGD₂ and PGE₂ inhibition. The findings supported augmentation of the COX-1 pathway by PGD₂, while an increase in PGE₂ contributed to the COX-2 pathway (43). Ketoprofen and indomethacin increased the tracheal susceptibility to acetylcholine and histamine; therefore, administration of these drugs is not recommended in

patients susceptible to allergy. Westerhof et al. (2001) reported that 24 h after guinea pigs were sensitized by ovalbumin, the total number of inflammatory cells such as lymphocytes, neutrophils, and especially eosinophils was increased in the serous membranes of lung tissue (44). An earlier investigation (45) reported results similar to Westerhof et al (2001).

Limitations of the study:

In this study, we used an organ bath method to evaluate the tissue responsiveness, but we did not execute a test *in-vitro*. The latter approach would have given a more specific and detailed results. Also, we did not evaluate the cyclooxygenase and lipoxygenase pathways or focus on a single pathway like COX-2.

Conflict of Interest: The authors declare no conflict of interest.

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References

1. Van Eerdewegh P, Little RD, Dupuis J, Del Mastro RG, Falls K, Simon J, et al. Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. *Nature*. 2002;418(6896):426.
2. Leclerc M, LAVOIE-LAMOUREUX A, LAVOIE JP. Heaves, an asthma-like disease of horses. *Respirology*. 2011;16(7):1027-46.
3. Stevenson DD. Diagnosis, prevention, and treatment of adverse reactions to aspirin and nonsteroidal anti-inflammatory drugs. *Journal of Allergy and Clinical Immunology*. 1984;74(4):617-22.
4. Tabarraei H, Hassan J, Parvizi MR, Golshahi H. Evaluation of the acute and sub-acute toxicity of the black caraway seed essential oil in Wistar rats. *Toxicology reports*. 2019;6:869-74.
5. Hamad AM, Sutcliffe AM, Knox AJ. Aspirin-induced asthma. *Drugs*. 2004;64(21):2417-32.
6. Gohil U, Modan A, Gohil P. Aspirin induced asthma-a review. *Global J Pharmacol*. 2010;4:19-30.
7. Medeiros R, Figueiredo CP, Pandolfo P, Duarte FS, Prediger RD, Passos GF, et al. The role of TNF- α signaling pathway on COX-2 upregulation and cognitive decline induced by β -amyloid peptide. *Behavioural brain research*. 2010;209(1):165-73.
8. Goetzl E. Lipid mediators of hypersensitivity and inflammation. *Middleton's Allergy Principles & Practice*. 1998.
9. Dwyer JH, Allayee H, Dwyer KM, Fan J, Wu H, Mar R, et al. Arachidonate 5-lipoxygenase promoter genotype, dietary arachidonic acid, and atherosclerosis. *New England Journal of Medicine*. 2004;350(1):29-37.
10. Obase Y, Matsuse H, Shimoda T, Haahtela T, Kohno S. Pathogenesis and management of aspirin-intolerant asthma. *Treatments in respiratory medicine*. 2005;4(5):325-36.
11. Szczeklik A. Mechanism of aspirin-induced asthma. *Allergy*. 1997;52(6):613-9.
12. Dahlén B, Nizankowska E, Szczeklik A, Zetterstrom O, Bochenek G, Kumlin M, et al. Benefits from adding the 5-lipoxygenase inhibitor zileuton to conventional therapy in aspirin-intolerant asthmatics. *American journal of respiratory and critical care medicine*. 1998;157(4):1187-94.
13. Anderson WH, O'Donnell M, Simko BA, Welton AF. An in vivo model for measuring antigen-induced SRS-A-mediated bronchoconstriction and plasma S RS-A levels in the guinea-pig. *British journal of pharmacology*. 1983;78(1):67-74.
14. Anderson W, Krzanowski J, Polson J, Szentivanyi A. Characteristics of histamine tachyphylaxis in canine tracheal smooth muscle. *Naunyn-Schmiedeberg's archives of pharmacology*. 1979;308(2):117-25.
15. HITCHCOCK M. STIMULATION OF THE ANTIGEN-INDUCED CONTRACTION OF GUINEA-PIG TRACHEA AND IMMUNOLOGICAL RELEASE OF HISTAMINE AND SRS-A FROM SENSITIZED GUINEA-PIG LUNG BY (2-ISOPROPYL-3-INDOLYL)-3 PYRIDYL KETONE (L8027) AND INDOMETHACIN. *British journal of pharmacology*. 1980;71(1):65-73.
16. de Boer J, Meurs H, Flendrig L, Koopal M, Zaagsma J. Role of nitric oxide and superoxide in allergen-induced airway hyperreactivity after the late asthmatic reaction in guinea-pigs. *British journal of pharmacology*. 2001;133(8):1235-42.
17. Artwohl J, Brown P, Corning B, Stein S. Report of the ACLAM task force on rodent euthanasia. *Journal of the American Association for Laboratory Animal Science*. 2006;45(1):98-105.
18. Mitchell HW, Adcock J. Potency of several non-steroidal antiinflammatory drugs on airways responses to histamine. *European journal of pharmacology*. 1987;141(3):467-70.
19. Smith N, Broadley KJ. Optimisation of the sensitisation conditions for an ovalbumin challenge model of asthma. *International immunopharmacology*. 2007;7(2):183-90.

20. Lowry O, Rosbrough A, Randall R. Lowry assay protocol. *J Biol Chem, Lond.* 1951;193:265.
21. Peebles S. Endogenous glucocorticoids and antigen-induced acute and late phase pulmonary responses. *Clinical & Experimental Allergy.* 2000;30(9):1257-65.
22. You HJ, Seo J-M, Moon J-Y, Han S-S, Ko Y-G, Kim J-H. Leukotriene Synthesis in Response to A23187 Is Inhibited by Methyl- β -Cyclodextrin in RBL-2H3 Cells. *Molecules & Cells (Springer Science & Business Media BV).* 2007;23(1).
23. Holtzman MJ, Ferdman B, Bohrer A, Turk J. Synthesis of the 1-O-hexadecyl molecular species of platelet-activating factor by airway epithelial and vascular endothelial cells. *Biochemical and biophysical research communications.* 1991;177(1):357-64.
24. Szczeklik A, Stevenson DD. Aspirin-induced asthma: advances in pathogenesis and management. *Journal of Allergy and Clinical Immunology.* 1999;104(1):5-13.
25. Sont JK, Willems LN, Bel EH, van KRIEKEN JHJ, Vandenbroucke JP, Sterk PJ, et al. Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guide to long-term treatment. *American journal of respiratory and critical care medicine.* 1999;159(4):1043-51.
26. Hashimoto K, Sheller JR, Morrow JD, Collins RD, Goleniewska K, O'Neal J, et al. Cyclooxygenase inhibition augments allergic inflammation through CD4-dependent, STAT6-independent mechanisms. *The Journal of Immunology.* 2005;174(1):525-32.
27. Newcomb DC, Peebles Jr RS. Th17-mediated inflammation in asthma. *Current opinion in immunology.* 2013;25(6):755-60.
28. Carey MA, Germolec DR, Langenbach R, Zeldin DC. Cyclooxygenase enzymes in allergic inflammation and asthma. Prostaglandins, leukotrienes and essential fatty acids. 2003;69(2-3):157-62.
29. Babu KS, Salvi SS. Aspirin and asthma. *Chest.* 2000;118(5):1470-6.
30. Sanjar S, Aoki S, Kristersson A, Smith D, Morley J. Antigen challenge induces pulmonary airway eosinophil accumulation and airway hyperreactivity in sensitized guinea-pigs: the effect of anti-asthma drugs. *British journal of pharmacology.* 1990;99(4):679-86.
31. Krzanowski J, Anderson W, Polson J, Szentivanyi A. Prostaglandin mediated histamine tachyphylaxis in subhuman primate tracheal smooth muscle. *Archives internationales de pharmacodynamie et de therapie.* 1980;247(1):155-62.
32. Szczeklik A, Nizankowska E, Bochenek G, Nagraba K, Mejza F, Swierczynska M. Safety of a specific COX-2 inhibitor in aspirin-induced asthma. *Clinical & Experimental Allergy.* 2001;31(2):219-25.
33. Kawaguchi A, Mizuta T, Matsuda H, Shirakura R, Nakahara K, Tanaka H, et al. Single lung transplantation in rats with chemically induced pulmonary hypertension. *The Journal of thoracic and cardiovascular surgery.* 1992;103(3):483-9.
34. Legen I, Kristl A. Ketoprofen-induced intestinal permeability changes studied in side-by-side diffusion cells. *Journal of pharmacy and pharmacology.* 2002;54(10):1419-22.
35. Santos A, Mingatto F, Curti C, Jordani M, Polizello A, Uyemura SA. In vitro interaction of nonsteroidal anti-inflammatory drugs on oxidative phosphorylation of renal cortex mitochondria respiration, atp synthesis, and induction of the membrane permeability transition. *Bollettino Chimico Farmaceutico.* 1997;136(2):65.
36. Paskauskas S, Parseliunas A, Kerkadze V, Nobiling R, Schmidt J, Ryschich E. Blockade of leukocyte haptokinesis and haptotaxis by ketoprofen, diclofenac and SC-560. *BMC immunology.* 2011;12(1):64.
37. Szczeklik A. Aspirin-induced asthma as a viral disease. *Clinical & Experimental Allergy.* 1988;18(1):15-20.
38. Nakagome K, Nagata M. Involvement and possible role of eosinophils in asthma exacerbation. *Frontiers in immunology.* 2018;9:2220.
39. Bloemen K, Verstraelen S, Van Den Heuvel R, Witters H, Nelissen I, Schoeters G. The allergic cascade: review of the most important molecules in the asthmatic lung. *Immunology letters.* 2007;113(1):6-18.

40. Asero R. Use of ketoprofen oral challenges to detect cross-reactors among patients with a history of aspirin-induced urticaria. *Annals of Allergy, Asthma & Immunology*. 2006;97(2):187-9.
41. García-Rodríguez R, Hinojosa M, Camacho-Garrido E, Berges Gimeno P, Martin Garcia C. Celecoxib, safe in NSAID intolerance. *Allergy*. 2002;57(11):1085-6.
42. Weberschock TB, Müller S-M, Boehncke S, Boehncke W-H. Tolerance to coxibs in patients with intolerance to non-steroidal anti-inflammatory drugs (NSAIDs): a systematic structured review of the literature. *Archives of dermatological research*. 2007;299(4):169-75.
43. Daham K, Song WL, Lawson J, Kupczyk M, Gülich A, Dahlén SE, et al. Effects of celecoxib on major prostaglandins in asthma. *Clinical & Experimental Allergy*. 2011;41(1):36-45.
44. Westerhof, F., Timens, W., Van Oosten, A., Zuidhof, A. B., Nauta, N., Schuiling, M, Coers, W. Inflammatory cell distribution in guinea pig airways and its relationship to airway reactivity. *Mediators of Inflammation*. 2001; 10(93): 143-154.
45. Underwood S, Foster M, Raeburn D, Bottoms S, Karlsson J. Time-course of antigen-induced airway inflammation in the guinea-pig and its relationship to airway hyperresponsiveness. *European Respiratory Journal*. 1995;8(12):2104-13.