Antiasthmatic effects of evening primrose oil in ovalbumin-allergic rats

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ABSTRACT

Asthma is one of the most common long-term conditions worldwide, which places considerable pressure on patients, communities and health systems. The uses of complementary and alternative medicines (CAMs) adjunctively to minimize the need for conventional therapies and hence avoid the profound side-effect profiles. Hence the effects of evening primrose oil (PO) alone or combined with dexamethasone (DEX) have been evaluated on bronchial asthma. We investigated the chronic effect of ovalbumin (OVA) in a rat model of allergic asthma. Rats were sensitized to OVA (1 mg/kg; i.p.) for 3 consecutive days, rats were pretreated orally with DEX (1 mg/kg), PO alone in three doses (1, 2 and 3 g/kg), PO (1.5 g/kg) combined with DEX (0.5 mg/kg) and saline 1 h before exposure to 1% OVA aerosol challenge (1 day/week for 3 weeks) then compared with positive control rats (OVA rats). Lung function tests were assessed after the last challenge and 24 h thereafter, blood films were prepared for assessment of eosinophil count and blood samples were collected for assessment of serum total protein as well as immunoglobulin E (Ig-E) levels. Lungs were isolated for histopathological study and also for determination of tumor necrosis factor-alpha (TNF-α) content in lung tissue. Additionally the effects of test agents were evaluated in acetyl choline (ACh)-induced airway constriction. PO alone and combined with DEX modulate Ig-E, TNF-α, eosinophil recruitment, airway constriction and remodelling. In conclusions, PO alone or combined with a lower dose of DEX possesses antiasthmatic effects.

Key words: asthma, evening primrose oil, dexamethasone, ovalbumin, acetylcholine

INTRODUCTION

The prevalence of allergic diseases has considerably increased, mostly in industrialized countries (> 20%), and asthma affects approximately 300 million individuals worldwide [1]. Asthma is a chronic inflammatory airway disease. It is characterized by airway hyperresponsiveness (AHR), multicellular inflammation, hypersensitive response of the immune system and airway obstruction and is accompanied by intermittent episodes of wheezing and coughing [2]. Early on, even in mild or moderate asthma, there is prominent thickening of a fibrillary layer subjacent to the epithelium [3]. However, its relevance to functional changes in asthma is unclear, and recent investigations suggest that subepithelial thickening may be a consequence of bronchoconstriction rather than a contributor to airflow obstruction [4]. Moreover the most important feature of asthmatic airway remodeling is thickening of the airway smooth muscle (ASM) layer [5]. This includes hypertrophy and hyperplasia of the muscle cells, as well as altered deposition of extracellular matrix (ECM) [6]. The poor response to treatment observed in patients with asthma may be a consequence of ongoing airway remodeling that result in fixed airway obstruction [7].
Glucocorticoid therapy is one of the most effective anti-inflammatory treatments available for asthma. This is likely due to multiple effects on the inflammatory response, including reduced production of cytokines and reduced antigen-induced infiltration of eosinophils [8]. Long-term administration of glucocorticoids has been shown to result in mitochondrial dysfunction as well as oxidative damage of mitochondrial and nuclear DNAs [9].

There is considerable interest in complementary and alternative medicine (CAM). These therapies include herbal preparations, mineral supplements, sugar restriction, and polyunsaturated fatty acids (PUFA) [10].

Evening primrose (Oenothera biennis L., Onagraceae) is a little plant with pretty yellow flowers blooming in the evening, related to rosebay willow herb family. Clinical trials on beneficial effects of its oil (PO) have been reported in atopic dermatitis[11].

PO is widely used as a dietary supplement due to its high content of n-6 PUFAs and gamma linoleic acid (GLA). GLA-containing oils such as PO is often used for the treatment of allergic symptoms or eczema [12] and used for several woman's health conditions, including breast pain (mastalgia), menopausal and premenstrual symptoms, cervical ripening, and labor induction [13].

The aim of this study the inflammatory, immunological features of asthma and the remodeling changes associated with chronic asthma as well as to evaluate the ability of PO to prevent the incidence of bronchial asthma in rats alone or combined with half the dose of dexamethasone (DEX) to minimize adverse effects associated with glucocorticoids.

MATERIALS AND METHODS

2.1. Animals

Adult male albino Wistar rats, weighing 120 – 140g each were used in the current study. They were purchased from the National Research Centre (NRC; Giza, Egypt). Animals received human care in compliance with the guidelines of the animal care and use committee of the NRC. The animals were kept in a quiet place and were allowed free access to water and standard food pellets throughout the period of investigation. Experiments were performed according to the National Regulations of Animal Welfare and Institutional Animal Ethical Committee (IAEC).

2.2. Chemicals

Ovalbumin (OVA; grade III), aluminum hydroxide and acetylcholine (ACh) were obtained from Sigma Aldrich Chemical Co. (USA). The chemicals, reagents and reagent kits used in the present study were of analytical grade.

2.3. Drugs

PO and DEX were obtained from T3A Co. and Sigma Co. (Egypt), respectively. PO was administered p.o. at a dose of 1, 2 & 3 ml/kg (equivalent to 1, 2 &3 g/kg) [14]. DEX was prepared in saline and administered p.o. at a dose of 1 mg/kg [15].

2.4. Experimental Design

The present study included evaluation of effects of PO and DEX in two experimental models of bronchial asthma and airway constriction.

2.4.1. Effect of evening primrose oil alone and combined with dexamethasone on ovalbumin-induced early and late airway reactions in rats.

Rats were randomly allocated into 7 groups (n=6). Asthma was induced by OVA sensitization followed by OVA challenge. First, animals were sensitized by i.p. injection of 1 mg/kg OVA/100 mg aluminum hydroxide suspended in 1 ml normal saline for 3 consecutive days. Three days after the final injection, the animals were challenged by exposure to 1% OVA for 15 min. Animals were challenged one day/week for 3 successive weeks by aerosolizing OVA solution contained in a specially devised plastic cylindrical chamber (200 ml capacity) introduced in an ultrasonic nebulizer (DEVILBISS ULTRA-NEB 99, 099HD) [16]. Induction of asthma was done in all groups except the 1st group: rats received saline instead of OVA to serve as normal control.

Test agents were orally administered 1 h before each OVA challenge as follows: 2nd group was left un-treated to serve as positive control. 3rd group: rats received DEX (1 mg/kg), 4th-6th : rats received PO (1, 2 and 3 g/kg),
respectively and 7th group rats received PO (1.5 g/kg) plus DEX (0.5 mg/kg). Assessment of early airway reaction (EAR) was performed 12 min after the last challenge by estimation of tidal volume (TV) and peak expiratory flow (PEF). Blood samples were collected, 24 h after the last challenge for blood films and for assessment of late airway reaction (LAR) by measurement of eosinophil count, serum total protein and immunoglobulin-E (IgE) levels. Moreover lungs were isolated for histopathological study as well as determination of TNF-α content in lung tissue.

2.4.2. Effect of evening primrose oil alone and combined with dexamethasone on peak expiratory flow in rats subjected to acetyl choline-induced airway constriction.

Rats were randomly allocated into 7 groups as above mentioned in OVA experiment. Each group consisted of 18 rats. All groups were subjected to cumulative inhalation of ACh (0.001%, 0.01% and 0.03%), each for 3 min using an ultrasonic nebulizer (DEVILBISS ULTRA-NEB 99, 099HD) except the 1st group: rats were exposed to saline aerosol and served as normal control group. 2nd group: rats were left untreated and served as positive control. From 3rd-7th groups, rats were given the test agents orally 20 min before ACh challenge. Assessment of PEF was performed using a spirometer immediately after ACh challenge [17].

2.5. Methods

2.5.1. Measurement of Tidal Volume and Peak Expiratory Flow

Rats were placed in a specific body plethysmograph made of plexi glass. Rats head protruded through a neck collar made of a dental latex dam into a head exposure chamber that ends with a flow head connected to spirometer (ADInstruments spirometer, ML140) which is a precision differential pressure transducer for measuring respiratory variables, such as TV and inspiration and expiration flows. It measures differential pressure across fine gauze mounted in a flow head.

2.5.2. Preparation of blood films, blood samples and lung homogenates

Blood films were done using cytospin slides and stained by Gimsa stain for counting eosinophil.

Blood samples (3 ml) were collected from the retro-orbital plexus vein of all rats. Samples were left to clot at room temperature then centrifuged at 1500 rpm for 10 min for serum separation. Serum samples were stored at -80°C for analysis of total protein and IgE levels.

Animals were then sacrificed by cervical dislocation and the two lungs were dissected and weighted separately. One lung was used for histopathological study and the other lung was homogenized in ice-cold phosphate buffer (pH 7.4) to prepare 20% w/v homogenate using a homogenizer (Heidolph, DIAx 900, Germany). Lung homogenates were centrifuged at 2000 xg for 20 min at 4 °C then stored at -80°C for analysis of TNF-α.

2.5.3. Biochemical measurements

Measurement of eosinophil count in blood was done using cytospin slide stained by Gimsa. A total of 200-300 cells were counted on each slide under x 500 magnification by oil immersion lens. Eosinophil count was expressed in blood as % of total white blood cells' count.

Determination of serum total protein was done based on biuret reaction and its level was expressed as g/dl. Serum IgE was determined by enzyme-linked immunosorbent assay (ELISA) using commercial kits (KOMA BIOTECH, Korea) and its level was expressed as ng/dl. Lung TNF-α was determined by ELISA using commercial kits (KOMA BIOTECH, Korea) and its level was expressed as pg/g wet tissue.

2.5.4. Histopathological study

Lung specimens of all animals were dissected immediately after sacrificed them, washed thoroughly with saline and fixed in 10% neutral-buffered formal saline for 72 h at least. All the specimens were washed in tap water for half an hour, dehydrated in ascending grades of alcohol (70% - 90% - 95% - absolute), cleared in xylene and then embedded in paraffin wax. Serial sections of 6 µm thick were cut and stained with haematoxylin and eosin for histopathological investigation [18].

2.5.5. Statistical analysis:

Data are expressed as mean ± S.E. Data analysis was done using one way analysis of variance (ANOVA) followed by least significant difference (LSD) test for multiple comparisons. Difference was considered significant when p is less than 0.05. SPSS (version 11) program was used to carry out these statistical tests.
RESULTS

3.1. Effect of evening primrose oil alone or combined with dexamethasone on Tidal Volume and Peak Expiratory Flow in asthmatic rats

OVA challenge significantly decreased TV and PEF to 27.95% and 30.70%, respectively as compared with the normal control group. Administration of DEX (1 mg/kg) 1h before each OVA challenge increased TV and PEF to 273.07% and 238.79%, respectively as compared with OVA group. Administration of PO (2 & 3 g/kg) significantly increased TV to 192.30%, and 269.23%, respectively, as well as increased PEF to 212.56% and 236.88%, respectively as compared with OVA group. Moreover combined administration of PO (1.5 g/kg) and DEX (0.5 mg /kg) increased TV and PEF to 280.76% and 245.08%, respectively as compared with OVA group (Table-1).

3.2. Effect of evening primrose oil alone or combined with dexamethasone on blood eosinophil count, serum total protein and immunoglobulin-E levels as well as lung content of tumor necrosis factor-alpha in asthmatic rats

OVA challenge significantly increased eosinophil count, serum levels of total protein and Ig-E as well as lung TNF-α content to 753.84%, 141.21%, 201.59% and 133.34%, respectively as compared with the normal control group. Prior administration of DEX 1h before each OVA challenge significantly decreased eosinophil count, total protein, Ig-E and TNF-α values to 24.48%, 74.52%, 57.27% and 74.90%, respectively as compared with OVA group. PO (1 g/kg) significantly decreased eosinophil count, serum total protein and Ig-E levels to 76.53%, 83.04%and 58.55%, respectively as compared with OVA group; meanwhile lung content of TNF-α was not affected. PO (2 & 3 g/kg) 1h before each OVA challenge significantly decreased eosinophil count to 62.24% and 50%; decreased serum total protein to 72.51% and 69.70%; decreased serum Ig-E to 53.44% and 50.88% and lung TNF-α to 84.10% and 82.28%, respectively as compared with OVA group. In addition combination of PO with DEX significantly decreased eosinophil count, total protein, Ig-E and TNF-α values to 19.38%, 73.01%, 49.85 % and 73.37%, respectively as compared with OVA group (Figure-1).

3.3. Effect of evening primrose oil alone or combined with dexamethasone on Peak Expiratory Flow in rats subjected to acetyl choline-induced airway constriction.

Cumulative inhalation of ACh (0.003- 0.03%), each for 3 min, produced a significant decrease in PEF to 79.36%, 62.47% and 39.58%, respectively as compared with the normal control group. Pretreatment with DEX increased PEF to 122.83%, 142.27% and 162.28%, respectively as compared with control ACh group. Administration of PO (2 & 3 g/kg; p.o.) 20 min before inhalation of ACh (0.003%) significantly increased PEF to 111.73% and 121.98%, respectively as compared to ACh group. Similarly administration of PO (2, 3 g/kg; p.o.) 20 min before ACh (0.01%) inhalation significantly increased PEF to 124.35% and 136.25%, respectively as compared to ACh group; whereas administration of the same doses before ACh (0.03%) inhalation increased PEF to 148.68% and 158.77%, respectively as compared to ACh group. Finally, administration of PO (1.5 g/kg; p.o.) combined with DEX (0.5 mg/kg; p.o.) 20 min before ACh (0.003 -0.03%) inhalation significantly increased PEF to 124.10%, 141.72% and 167.54%, respectively as compared with ACh group (Figure-2).

3.4. Effect of evening primrose oil alone or combined with dexamethasone on lung histopathological changes in asthmatic rats

Light microscopic examination of lung tissue section obtained from a normal rat showed normal wall of a bronchiole with its lining epithelium (pseudostratified epithelium) lying on a thin layer of smooth muscle fibers (Figure-3A). Lung tissue section of asthmatic rat showing marked localized cellular infiltration in the submucosa of the bronchiole (green arrow head), discontinuation of the smooth muscle layer (black arrow head) and hypertrophy of the epithelial lining. The upper right corner of the figure is a higher magnification (X 200) showing eosinophils in the cellular infiltration (arrow) (Figure-3B). Lung tissue section of a rat pretreated with DEX (1 mg/kg) showed slight interstitial cellular infiltration (arrow head) with no thickening of smooth muscle layer in the bronchiolar wall (Figure-3C). Lung tissue section of a rat treated with PO (1 g/kg) showing fibrosis (arrow), cellular infiltration (arrow head) and hypertrophy of the epithelial lining of the bronchiolar wall (green arrow head) (Figure-3D). Lung tissue section of a rat treated with PO (2 g/kg) showed cellular infiltration but to a lesser degree (Figure-3E). Treatment with PO (3 g/kg) resulted in both the alveolar septae and the bronchiolar wall appear more or less normal (Figure-3F). The combination of both DEX and PO resulted in minimal histopathological changes in lung tissues (Figure-3G).
Table (1): Effect of evening primrose oil (PO) alone or combined with dexamethasone (DEX) on tidal volume (TV) and peak expiratory flow (PEF) in asthmatic rats

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>Normal control</th>
<th>OVA</th>
<th>DEX (1mg/kg)</th>
<th>PO (1g/kg)</th>
<th>PO (2g/kg)</th>
<th>PO (3g/kg)</th>
<th>PO (1.5 g/kg) + DEX(0.5mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TV (ml)</td>
<td>0.093±0.001</td>
<td>0.071±0.001*</td>
<td>0.033±0.001*</td>
<td>0.050±0.002*</td>
<td>0.070±0.002*</td>
<td>0.073±0.001*</td>
<td></td>
</tr>
<tr>
<td>PEF (ml/min)</td>
<td>11.92 ± 0.28</td>
<td>3.66 ± 0.12*</td>
<td>8.74 ± 0.12*</td>
<td>4.44 ± 0.15*</td>
<td>7.78 ± 0.12*</td>
<td>8.67 ± 0.06*</td>
<td>8.97 ± 0.01*</td>
</tr>
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Asthma was induced by i.p. administration of ovalbumin (OVA; 1mg/kg) for 3 consecutive days then OVA inhalation (1%) one day/week for 3 weeks. Drugs were orally administered 1 h before each OVA challenge. Measurements were carried out 12 min after the last challenge. Data were expressed as mean ± SE (n=6). Statistical analysis was carried out by one-way analysis of variance followed by Least Significant Difference test. *Significantly different from normal control at p<0.05. †Significantly different from OVA group at p<0.05.

Figure (1): Effect of evening primrose oil (PO) alone or combined with dexamethasone (DEX) on: A. eosinophil count, serum levels of B. total protein and C. immunoglobulin-E (Ig-E) as well as lung content of D. tumor necrosis factor-alpha (TNF-α) in asthmatic rats. Asthma was induced by i.p. administration of ovalbumin (OVA; 1mg/kg) for 3 consecutive days then OVA inhalation (1%) one day/week for 3 weeks. Drugs were orally administered 1 h before each OVA challenge. Blood film and samples as well as tissue samples were collected 24h after the last challenge. Data were expressed as mean ± SE (n=6). Statistical analysis was carried out by one-way analysis of variance followed by Least Significant Difference test. *Significantly different from normal control at p<0.05. †Significantly different from OVA group at p<0.05.
Figure (2): Effect of evening primrose oil (PO) alone or combined with dexamethasone (DEX) on acetyl choline (ACh)-induced airway constriction in rats

Airway constriction was induced by cumulative inhalation of ACh (0.003-0.03%; each for 3 min). Drugs were orally administered 20 min before ACh challenge. Measurement of peak expiratory flow (PEF) was carried out immediately after ACh challenge. Data were expressed as mean ± SE (n=6). Statistical analysis was carried out by one-way analysis of variance followed by Least Significant Difference test. *Significantly different from normal control at p<0.05. **Significantly different from ACh group at p<0.05.
DISCUSSION

In previous study, we found that OVA sensitizing followed by challenge with OVA for periods of 3 weeks was used as a model of chronic asthma and increased airway responsiveness as well as increased eosinophil infiltration into the airways in rats [14]. In the current work, we studied the effects of PO alone or with lower dose of DEX. OVA challenge (1%) significantly decreased TV and PEF, as compared with normal control group, indicating constriction of airway smooth muscle. These changes are in harmony with those of another study [16]. While DEX administration (1mg/kg; p. o.; 1 h before OVA challenge) significantly increased TV and PEF, respectively, as compared with OVA group. A similar pattern was reported [19], when rats treated i.p with DEX (300 µg/kg) 14 and 2 h before OVA challenge. Moreover inhaled budesonide (2.5 mg/kg) 18 and 1 h before OVA challenge inhibited OVA-induced airway narrowing [8].

Figure (3): Photomicrographs of sections of the lung tissue of: (A) A normal rat; (B) Asthmatic rat; (C) DEX (1 mg/kg)-treated rat; (D) PO (1 g/kg)-treated rat; (E) PO (2 g/kg)-treated rat; (F) PO (3 g/kg)-treated rat and (G) PO (1.5 mg/kg)- and DEX (0.5 mg/kg)-treated rat. (Hematoxylin and eosin X 50)
In the last decades there has been an increase in allergic disease throughout the world, particularly in children. Attempts have been made to identify the causes of this "allergy epidemic" in environmental changes and changes in population hygiene, lifestyle, socioeconomic level, and eating habits that would exert epigenetic effects. A major contributing factor in asthma was dietetic changes associated with the transition from a traditional to a modern diet, in which foods are processed to allow for longer preservation, and salt, refined sugar and saturated fat consumption has increased coupled with a decrease in the consumption of fruit, vegetables, milk (which moreover is consumed in ultra-pasteurised form), dietary fiber as well as foods rich in vitamins and antioxidants. Dietetic hypotheses have been mainly focused on long-chain polyunsaturated fatty acids [20].

In present work, PO (rich in n-6 PUFAs) in the higher dose levels (2-3 g/kg) significantly attenuated OVA-induced decrease in TV and PEF. Similar findings were noted also when PO was combined with half the dose of DEX. The present data are in line with previous studies on borage oil, rich in n-6 PUFAs as PO that showed modulation in lung compliance and oxygenation in acute respiratory distress syndrome [21].

Eosinophils play a pivotal role in the pathophysiology of asthma as they are sources of cytokines. In addition, eosinophils are responsible for the changes in airway submucosal tissue resulting in airway responsiveness [22]. In current results, OVA challenge induced significant increase in eosinophil count as compared with the normal control group. This is in line with previous result [23]. While DEX administration, in this study, significantly decreased eosinophil count, as compared with OVA group. Inhaled budesonide, in another study, abolished the late response to OVA [8]. Also, PO administration alone or combined with half the dose of DEX significantly attenuated the increase in eosinophil count induced by OVA. Similar results was observed with administration of omega-3 PUFA in experimentally-induced asthma in cats and rats [24].

In present study, OVA challenge produced a significant increase in serum total protein indicating plasma extravasation, an established feature in experimental models of asthma [25]. The present increase in total protein was supported by histopathological examination of control asthmatic group that showed vascular leakage. While DEX administration significantly decreased serum total protein, as compared with OVA group. Such effect was supported by histopathological results of DEX group that showed reduction in vascular leakage.

In addition, PO (1, 2, 3 g/kg) significantly attenuated OVA-induced increase in serum total protein. Similar results were noted when PO was combined with half the dose of DEX. Moreover, the histopathological study showed that PO in highest dose level (3 g/kg) or combined with half the dose of DEX showed inhibition of microvascular leakage which suggested that PO alone or combined with half the dose of DEX have antiexudative effect against OVA challenge via reducing the airway microvascular leakage. This result correlate with that obtained using fish oil rich in n-3 PUFAS [14].

Results of the current study revealed that OVA challenge produced a significant increase in serum Ig-E level. This increase was observed also in another study using OVA intranasal to induce asthma [26]. The administration of DEX, in present work, prevented the increase in serum Ig-E level and mentioned in previous study on DEX that showed a significant attenuation in dermatophagoides farina (dust mite)-induced increase in serum Ig-E [15]. Administration of PO (1, 2, 3 g/ kg) significantly attenuated OVA-induced increase in serum IgE level. Same results were found when PO was given combined with half the dose of DEX which indicating that PO alone or combined with half the dose of DEX may posses anti-IgE effect through modifying the patterns of cytokines produced by Th cells. This result is in a harmony with a study which proved that PO reduced serum Ig-E level in atopic dermatitis[27].

In the present results, OVA challenge significantly increased lung content of TNF-α, a proinflammatory cytokine implicated in the pathogenesis of asthma [28]. Histopathological examination of lung tissues of control asthmatic group revealed severe cellular eosinophils infiltration. Presence of eosinophils together with increased lung TNF-α content suggests the important role played by the latter in the initial phase of the inflammatory response as well as the late-phase airway response and cell recruitment. This is consistent with previous study [29].

Our data revealed that DEX administration significantly decreased lung TNF-α content, as compared with OVA group. Similar results were previously reported [30]. Therapy with glucocorticoids is considered the most effective anti-inflammatory treatment available for asthma. This is likely to be due to multiple effects on the inflammatory

response, including inhibition in leukocyte migration into sites of inflammation [31] and reduction in cytokines production [32].

PO in the higher dose levels (2-3 g/kg) attenuated OVA-induced increase in lung TNF-α content. The same effect was noted when PO was combined with half the dose of DEX. In addition, the histopathological study of PO (3 g/kg) showed a great reduction of interstitial cellular infiltration. Moreover, both the alveolar septa and the bronchiolar wall appeared more or less normal, supporting that PO has an additional factor, beside TNF-α, that is the control of late eosinophil recruitment into the lung during allergic inflammation. These results are in agreement with previous study using borage oil rich in n-6 PUFAs that showed modulation of lung pulmonary inflammation in acute respiratory distress syndrome [21].

n-6 PUFAs, present in PO, reduced the inflammation by a different mechanism when compared to n-3 PUFAs, as n-6 is metabolized to dihomo gama linolenic acid(DGLA), that is a substrate for series-1 PGs and TXs, which in turn elevates PGE1 and TXA1. PGE1 suppresses synthesis of LT4, a pro-inflammatory mediator, and suppresses chronic inflammation. TXA1 modulates the pro-inflammatory properties of the thromboxane (TXA2). Unlike other eicosanoids, DGLA cannot yield leukotrienes. However, it can inhibit the formation of pro-inflammatory leukotrienes from arachidonic acid. This anti-inflammatory role of GLA has been utilized in the treatment of various diseases involving diverse systems [11].

In this study, cumulative inhalation of ACh (0.003- 0.03 %), each for 3 min, produced a significant decrease of PEF, as compared with the normal control group suggesting airway constriction that is one of the most important factors in the asthmatic reaction. This result is confirmed by other study [17]. While DEX administration (1 mg/kg) 20min before ACh inhalation increased PEF. Inhibition of adenosine monophosphate-induced airway constriction by DEX was previously reported [33]. Also, PO in the higher dose levels (2-3 g/kg) significantly attenuated ACh-induced decrease in PEF. Similar finding were observed when PO was combined with half the dose of DEX. Reduction in airway narrowing following ACh exposure by fish oil supplement was previously reported [14].

Indeed the present results revealed that a number of structural changes were observed in OVA group including cellular infiltration, interstitial hemorrhage, discontinuation of the smooth muscle layer and hypertrophy of the epithelial lining. Such changes therefore may likely represent the early changes occuring in the course of airway remodeling. Similar findings were reported in other study [34].

Histopathological examination showed that DEX or PO (3 g/kg) alone or combined with half the dose of DEX administration reduced signs of remodelling observed in the OVA group

CONCLUSION

PO alone or combined with lower doses of steroids abolished the OVA-induced early and late response as well as the associated increase in IgE and eosinophil infiltration in rats. In addition, it is possible that PO alone or combined with lower doses of steroids could reduce the responsiveness of the airways to ACh. So it may be possible to use PO alone or combined with lower doses of steroids as a safe and effective mean of asthma management and to minimize associated adverse effects of long-term use of steroids.

Acknowledgment

The authors are very grateful to Dr. Nermeen M. Shaffie, Assistant Professor of Pathology, National Research Centre, for examining and interpreting histopathological aspects of this study.

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