

Effects of Fish oil and Dexamethasone in Experimentally-Induced Bronchial Asthma

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Abstract: Background: Asthma is characterized by airway hyper-responsiveness, inflammation, obstruction and pathological remodelling. Objectives: To evaluate the efficacy of fish oil (FO) alone or combined with half the dose of dexamethasone (DEX) in experimentally-induced bronchial asthma. Material and Methods: Rats were allocated into 7 groups. Group 1 exposed to saline aerosol (normal control). Asthma was induced in the remaining groups by ovalbumin (OVA) sensitization (1 mg/kg OVA; i.p.) for 3 consecutive days followed by 1% OVA challenge (1 day/week for 3 weeks). One group was left untreated (positive control). In the remaining groups, test agents were orally administered 1 h before each OVA challenge as follows: group 3 received dexamethasone (DEX; 1 mg/kg), groups 4-6: received FO (1, 2 and 3 g/kg) and group 7 received FO (1.5 g/kg) plus DEX (0.5 mg/kg). Lung function tests were assessed 12 min after the last OVA 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 assessment and determination of tumor necrosis factor-alpha (TNF- α) content. Additionally the effects of test agents were evaluated in acetyl choline (ACh; 0.003-0.03%-induced airway constriction. Results: FO alone and combined with DEX attenuated OVA-induced changes in lung function tests, reduced OVA-induced increase in eosinophil count, serum total protein and Ig-E levels as well as lung TNF- α content and reduced airway remodelling. Moreover, FO and DEX inhibited ACh-induced airway constriction. Conclusions: FO can be used alone or combined with a lower dose of DEX in treatment of bronchial asthma.

Key words: Asthma, Fish oil, Dexamethasone, Ovalbumin, Acetylcholine.

INTRODUCTION

The prevalence of asthma has dramatically increased in the last decades. According to the latest report of World Health Organization about 300 million people worldwide have suffered from asthma and 255,000 died from asthma (Wang, *et al.*, 2012).

Asthma is a chronic disease characterized by inflammation of the airways associated with a hypersensitive response of the immune system (Ohmori *et al.*, 2002). Development of asthma passes through three phases. During the induction phase, Activation of T-helper cells leads to the production of various cytokines, as tumor necrosis factor-alpha (TNF- α), interleukins and interferons (Kay, 2003). Early-phase asthmatic reaction (EAR) is characterized by constriction of airway smooth muscle (ASM) cells, vascular leakage, mucus production and recruitment of inflammatory cells (Bradding and Holgate, 1999). Late phase asthmatic reaction (LAR) is characterized by excessive inflammation of the airways resulting in structural changes known as airway remodeling that includes airway wall thickening, subepithelial fibrosis, ASM and epithelial cells hypertrophy (Bloemen *et al.*, 2007).

Complementary and alternative medicines (CAMs) are used in more than 80% of the world's population and are becoming an important component in health care systems all over the world (Mainardi *et al.*, 2009). Advances in dietary oil research have indicated that increased consumption of long chain omega-3 polyunsaturated fatty acids, found in fish oil (FO), may be of therapeutic value in a variety of acute and chronic inflammatory conditions (Calder, 2006). Omega-3 fatty acids are postulated to have a protective role in the development of inflammatory diseases such as diabetes mellitus, cardiovascular disease, rheumatoid arthritis and asthma (Wong, 2005).

Glucocorticoid therapy is one of the most effective anti-inflammatory treatment 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 (Xu *et al.*, 2000). Long-term administration of glucocorticoids has been shown to result in mitochondrial dysfunction as well as oxidative damage of mitochondrial and nuclear DNAs (Gvozdjáková *et al.*, 2005).

Accordingly, the present study aimed to explore the benefits of using FO in experimentally-induced 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 Center (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:

FO and DEX were obtained from Sedico Co. and Sigma Co. (Egypt), respectively. FO was administered p.o. at a dose of 1 ml/kg (equivalent to 1g/kg) (Gu *et al.*, 1998). DEX was prepared in saline and administered p.o. at a dose of 1 mg/kg (Xie *et al.*, 2008).

2.4. Experimental Design

The present study included evaluation of effects of FO and DEX in two experimental models of airway responsiveness.

2.4.1. Effect of Fish oil Alone or 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) (Salmon *et al.*, 1999). Induction of asthma was done in all groups except the 1st one, in which saline was used instead of OVA to serve as normal control.

Test agents were orally administered 1 h before each OVA challenge as follows: group 3 received DEX (1 mg/kg), groups 4-6 received FO (1, 2 and 3 g/kg), respectively and group 7 received FO (1.5 g/kg) plus DEX (0.5 mg/kg). Group 2 was left un-treated to serve as positive control.

Assessment of EAR was performed 12 min after the last challenge by estimation of tidal volume (TV) and peak expiratory flow (PEF). Blood films were made and blood samples were collected, 24 h after the last challenge, for assessment of LAR by measurement of eosinophil count, serum total protein and immunoglobulin-E (IgE) levels. Moreover lungs were isolated for histopathologica studies as well as determination of TNF- α content. The experimental design is illustrated in Figure (I).

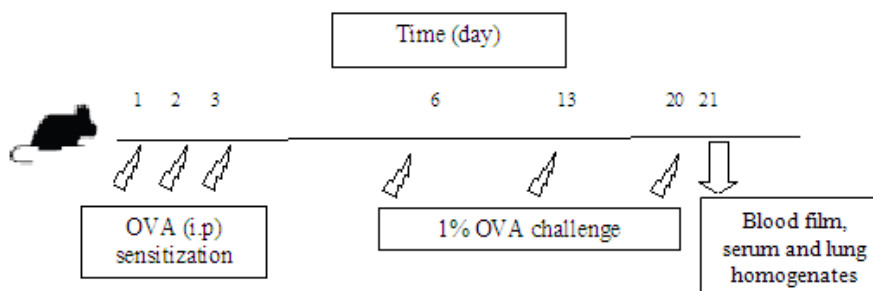


Fig. I: Experimental design of ovalbumin (OVA)-induced asthma.

2.4.2. Effect of Fish 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 (in a similar fashion as OVA experiment). Each group consisted of 18 rats (6 rats for each ACh concentration). Airway constriction to ACh was done according to the method described by Misawa and Chiba (1993). 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 one which was exposed to saline aerosol to serve as normal control group. Group 2 was left un-treated to serve as positive control. In groups 3-7, the test agents were administered p.o. 20 min before ACh challenge. Assessment of PEF was performed using a spirometer immediately after ACh challenge. The experimental design is illustrated in Figure (II).

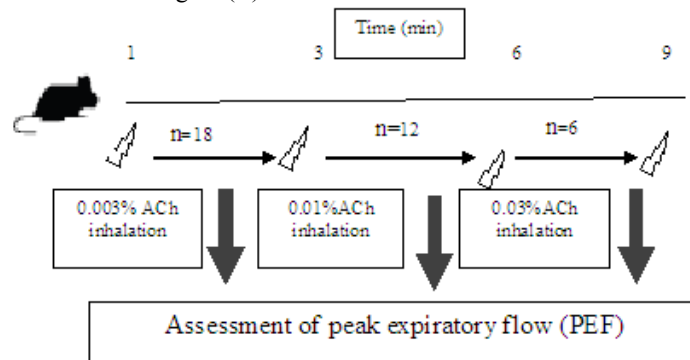


Fig. II: Design of acetyl choline (ACh)-induced airway constriction.

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 inspiration and expiration flows and TV. 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 made from cytopsin slides and stained by Gemsa 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 examination 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 made using cytopsin slide stained by Gemsa. 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 according to Gornal *et al.* (1949) 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 death, 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 μ m thick were cut and stained with haematoxylin and eosin for histopathological investigation.

2.5.5. Statistical Analysis:

Data are expressed as mean \pm 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.

3. Results:

3.1. Effect of Fish 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 FO (2 & 3 g/kg) significantly increased TV to 188.46% and 261.53%, respectively, as well as increased PEF to 212.56% and 236.61%, respectively as compared with OVA group. Moreover combined administration of FO (1.5 g/kg) and DEX (0.5 mg /kg) increased TV and PEF to 273.07% and 244.26%, respectively as compared with OVA group (Table-1).

3.2. Effect of Fish 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. FO (1 g/kg) significantly decreased eosinophil count, serum total protein and Ig-E levels to 77.55%, 88.66% and 81.06%, respectively as compared with OVA group; meanwhile lung content of TNF- α was not affected. FO (2 & 3 g/kg) 1h before each OVA challenge significantly decreased eosinophil count to 63.26% and 52.04%; decreased serum total protein to 85.45% and 79.53%; decreased serum Ig-E to 75.95% and 73.65% and lung TNF- α to 82.28% and 80.27%, respectively as compared with OVA group. In addition combination of FO with DEX significantly decreased eosinophil count, total protein, Ig-E and TNF- α values to 20.40%, 78.93%, 52.67% and 69.73%, respectively as compared with OVA group (Figure-1).

3.3. Effect of Fish 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 FO (2 & 3 g/kg; p.o.) 20 min before inhalation of ACh (0.003%) significantly increased PEF to 110.14% and 119.87%, respectively as compared to ACh group. Similarly administration of FO (2, 3 g/kg; p.o.) 20 min before ACh (0.01%) inhalation significantly increased PEF to 128.59% and 140.90%, respectively as compared to ACh group; whereas administration of the same doses before ACh (0.03%) inhalation increased PEF to 140.35% and 152.19%, respectively as compared to ACh group. Finally, administration of FO (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 121.98% and 140.76%, 161.84%, respectively as compared with ACh group (Figure-2).

3.4. Effect of Fish oil Alone or Combined with Dexamethasone on Lung Histopathologic 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 showed severe cellular infiltration, recruitment of eosinophils and marked interstitial hemorrhage (Figure-3B). Lung tissue section of a rat pretreated with DEX (1 mg/kg) showed dilatation and congestion of blood vessels together with slight thickening of alveolar septae (Figure-3C). Lung tissue section of a rat treated with FO (1 g/kg) revealed marked dilatation and congestion of blood vessels together with extravasation of blood in the lumen of the bronchiole and marked cellular infiltration in the interstitial spaces between bronchioles (Figure-3D). Lung tissue section of a rat treated with FO (2 g/kg) showed cellular infiltration but to a lesser degree (Figure-3E). Treatment with FO (3 g/kg) resulted in marked reduction in cellular infiltration as well as mild thickening in alveolar septae and normal epithelial lining of the bronchioles (Figure-3F). The combination of both DEX and FO resulted in minimal histopathologic changes in lung tissues (Figure-3G).

Table 1: Effect of fish oil (FO) alone or combined with dexamethasone (DEX) on tidal volume (TV) and peak expiratory flow (PEF) in asthmatic rats.

Groups	Normal control	OVA	DEX (1mg/kg)	FO (1g/kg)	FO (2g/kg)	FO (3g/kg)	FO (1.5 g/kg) + DEX (0.5mg/kg)
Parameters							
TV (ml)	0.093 ± 0.001	0.026 ± 0.001 ^a	0.071 ± 0.001 ^{ab}	0.032 ± 0.001 ^a	0.049 ± 0.002 ^{ab}	0.068 ± 0.001 ^{ab}	0.071 ± 0.001 ^{ab}
PEF (ml/min)	11.92 ± 0.28	3.66 ± 0.12 ^a	8.74 ± 0.12 ^{ab}	4.46 ± 0.21 ^a	7.78 ± 0.12 ^{ab}	8.66 ± 0.12 ^{ab}	8.94 ± 0.18 ^{ab}

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. ^aSignificantly different from normal control at $p < 0.05$. ^bSignificantly different from OVA group at $p < 0.05$.

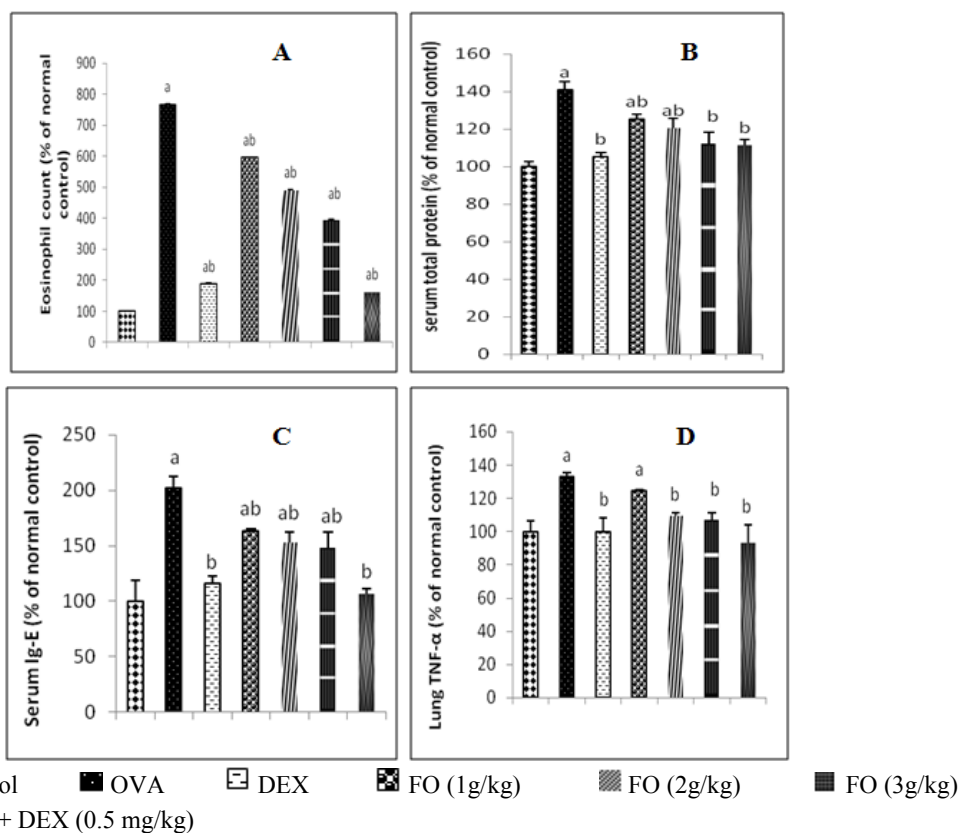


Fig. 1: Effect of fish oil (FO) alone or combined with dexamethasone (DEX) on: A. eosinophil count, serum levels of B. total protein and C. immunoglobulin-E (Ig-E) as well as lung content of D. tumor necrosis factor-alpha (TNF- α) in asthmatic rats.

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. ^aSignificantly different from normal control at $p < 0.05$. ^bSignificantly different from OVA group at $p < 0.05$.

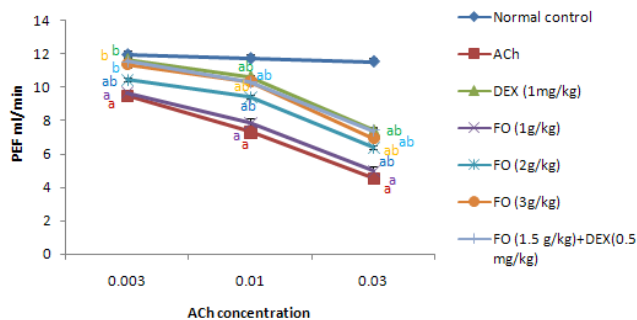


Fig. 2: Effect of fish oil (FO) alone or combined with dexamethasone (DEX) on acetyl choline (ACh)-induced airway constriction in rats.

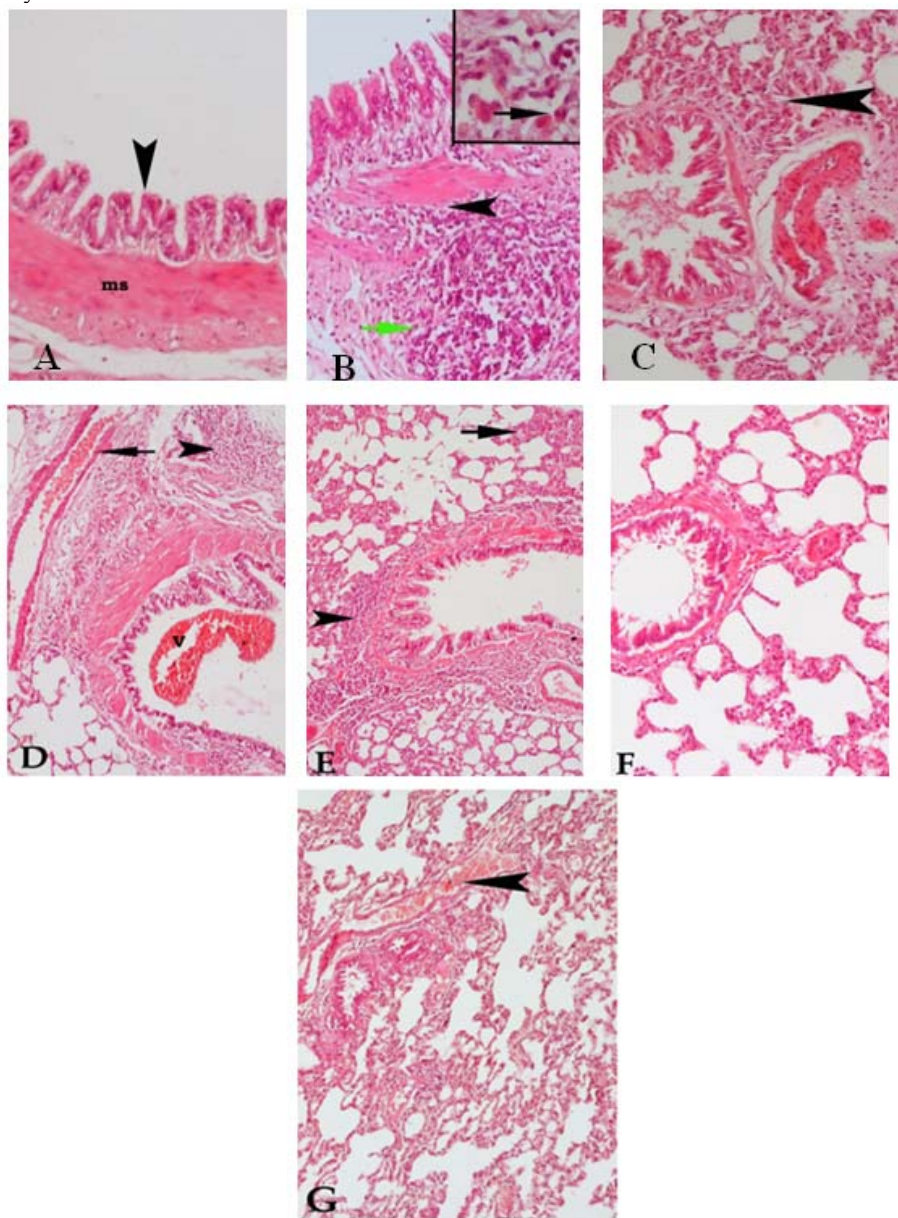


Fig. 3: Photomicrographs of sections of the lung tissue of: (A) A normal rat showing the wall of a bronchiole with its lining epithelium (pseudostratified epithelium)(arrow head) lying on a thin layer of smooth muscle fibres (ms); (B) Asthmatic rat showing marked localized cellular infiltration in the 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); (C) DEX (1 mg/kg)-treated rat showing slight interstitial cellular infiltration (arrow head) with no thickening of smooth muscle layer in the bronchiolar wall; (D) FO (1 g/kg)-treated rat showing marked dilatation and congestion of blood vessels (arrow), extravasation of blood in the lumen of the bronchioles (V). Marked cellular infiltration is observed in the wall of the bronchiole and in the interstitial spaces (arrow head); (E) FO (2 g/kg)-treated rat showing cellular infiltration in the bronchiolar wall (arrow head), but to a lesser degree with thickening of the alveolar septae in some areas (arrow); (F) FO (3 g/kg)-treated rat showing marked reduction in cellular infiltration as well as mild thickening in alveolar septae and normal epithelial lining of the bronchiole and (G) FO (1.5 mg/kg)- and DEX (0.5 mg/kg)-treated rat showing mild thickening of alveolar septae. (Hematoxylin and eosin X 50).

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 \pm SE (n=6). Statistical analysis was carried out by one-way analysis of variance followed by Least Significant Difference test. ^aSignificantly different from normal control at $p < 0.05$. ^bSignificantly different from ACh group at $p < 0.05$.

Discussion:

In the present study, OVA sensitization followed by OVA challenge was used as a model of chronic asthma in rats to investigate benefits of using FO alone or with lower dose of DEX. Sensitization followed by inhalational exposure to OVA is known to increase airway responsiveness and increase inflammatory cell infiltration into the airways (Elwood *et al.*, 1991).

In acute and subacute models of asthma, rats received a single sensitizing dose of OVA, followed by exposure to nebulized OVA solution for a short period of 3–4 or 7–10 days, respectively (Wegmann and Renz, 2005). In chronic models of asthma, rats are sensitized to OVA, often by two sensitizing doses, and then exposed to inhalational challenge with OVA for periods of 2–12 weeks (Kumar and Foster, 2001).

The current results revealed that OVA challenge (1%) significantly decreased TV and PEF, as compared with normal control group, indicating constriction of airway smooth muscle. The present changes are in harmony with those of Salmon *et al.* (1999).

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 recorded with i.p. treatment of rats with DEX (300 μ g/kg) 14 and 2 h before OVA challenge (Powell *et al.*, 1995). Moreover inhaled budesonide (2.5 mg/kg) 18 and 1 h before OVA challenge inhibited OVA-induced airway narrowing (Xu *et al.*, 2000). Glucocorticoids as DEX are often used as reference standard in models of allergic asthma (Das *et al.*, 1997).

FO in the higher dose levels (2-3 g/kg) significantly attenuated OVA-induced decrease in TV and PEF. Similar findings were noted when FO was combined with half the dose of DEX. The present data are in agreement with previous studies on Perilla seed oil which contain long chain omega-3 polyunsaturated fatty acids as in FO. Supplementation of the latter to asthmatic subjects increased PEF when compared with subjects treated with other oils as corn oil (Okamoto *et al.*, 2000).

In present data, OVA challenge produced a significant increase in eosinophil count as compared with the normal control group. Similar findings were reported by Bundschuh *et al.* (2001).

DEX administration significantly decreased eosinophil count, as compared with OVA group. This is consistent with previous investigation using inhaled budesonide that showed abolishment in the late response to OVA (Xu *et al.*, 2000).

FO administration alone or combined with half the dose of DEX significantly attenuated the increase in eosinophil count induced by OVA. A similar pattern was observed with omega-3 PUFA in experimentally-induced asthma in cats (Leemans *et al.*, 2010).

In our study, OVA challenge produced a significant increase in serum total protein indicating plasma extravasation, an established feature in experimental models of asthma Olivenstein *et al.* (1997). The present increase in total protein was supported by histopathological examination of control asthmatic group that showed vascular leakage.

The current results revealed that DEX administration significantly decreased serum total protein, as compared with OVA group. Such effect was supported by histopathological examination of DEX group where reduction in vascular leakage was noted. Similar finding was reported by Corrigan (2012).

FO (1, 2, 3 g/kg) significantly attenuated OVA-induced increase in serum total protein. Similar findings were noted when FO was combined with half the dose of DEX. In addition, the current histopathological study showed that FO in highest dose level (3 g/kg) or combined with half the dose of DEX inhibited microvascular leakage suggesting that FO alone or combined with half the dose of DEX have antiexudative effect against OVA challenge via reducing the airway microvascular leakage. The above findings correlate with that obtained by de

Matos *et al.* (2012) who stated that edema and eosinophil infiltration were significantly reduced in mice fed with diet rich in n-3 PUFAS.

Results of the present work revealed that OVA challenge produced a significant increase in serum Ig-E level. Similar results were previously reported (Haczku *et al.*, 1995).

In the current study, OVA-induced increase in serum Ig-E level was prevented by administration of DEX. The present data are in agreement with previous study on DEX that showed a significant attenuation in dermatophagoides farina (dust mite)-induced increase in serum Ig-E (Xie *et al.*, 2008).

FO (1, 2, 3 g/ kg) significantly attenuated OVA-induced increase in serum Ig-E. Similar findings were noted when FO was combined with half the dose of DEX indicating an anti-IgE effect for FO possibly by modifying the patterns of cytokines produced by Th cells. These results are in accordance with other study using n-3 PUFAs in an experimental model of food allergy with OVA diet (de Matos *et al.*, 2012).

In the present study, OVA challenge significantly increased lung content of TNF- α , a proinflammatory cytokine implicated in the pathogenesis of asthma through amplifying the inflammatory response in asthma.

Previous studies showed that OVA can increase the production of tissue TNF- α , which in turn activates nuclear factor kappa (NF- κ B). NF- κ B crosses the nuclear membrane and activates several genes within the nucleus resulting in increased prostaglandin G₂ synthesis via cyclo-oxygenase (COX) pathway leading to inflammation (Barnes and Adcock, 1998).

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 the study of Lukacs *et al.* (1995) who stated that TNF- α mediates the recruitment of neutrophils and eosinophils during antigen-induced airway inflammation.

Results of the current study revealed that DEX administration significantly decreased lung TNF- α content, as compared with OVA group. Similar results were previously reported (Renzi *et al.*, 1993; Xu *et al.*, 2000). 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 (Schleimer, 1990) and reduction in cytokines production (Barnes, 2002).

In a similar fashion, administration of FO in doses of 2 and 3 g/kg or its combination with a lower dose of DEX significantly decreased lung TNF- α content. Effect of FO on TNF- α content was supported by histopathological examination of FO (3 g/kg)-treated group where cellular infiltration and pulmonary eosinophilia were inhibited.

The present data are in agreement with another study using perilla (n-3 series) that related the anti-inflammatory potential of perilla to suppression of production of serum PGE₂ and LTB₄ due to the reduction in arachidonic acid (AA) level (Gu *et al.*, 1998). Moreover n-3 PUFAS inhibited the 5- lipooxygenase pathway and LTB₄ production in neutrophils, in vitro experiments (Lee *et al.*, 1985). Hence, the observed anti-inflammatory potential of FO could be mediated through suppression of the production of AA metabolites by two mechanisms, reducing the substrate level and inhibiting the enzymatic activity owing to its high content of n-3 PUFAS.

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 Misawa and Chiba (1993).

The current work proved that DEX administration (1 mg/kg) 20min before ACh inhalation increased PEF. Inhibition of adenosine monophosphate-induced airway constriction by DEX was previously reported (Taylor *et al.*, 1999).

FO in the higher dose levels (2-3 g/kg) significantly attenuated ACh-induced decrease in PEF. Similar finding were observed when FO was combined with half the dose of DEX. Reduction in airway narrowing following exercise by FO supplements was previously reported (Mickleborough and Rundell, 2005).

Clinical studies suggested that remodeling occurs in early life, before the clinical manifestation of asthma, as a result of a primary defect of the epithelium and epithelial repair process (Pohunek *et al.*, 1997). 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 occurring in the course of airway remodeling. Similar findings were reported by Spinelli *et al.* (2012).

Histopathological examination showed that DEX administration reduced signs of remodelling observed in the control group. Corticosteroids strongly inhibit T cell pro-inflammatory cytokine production and therefore reduce infiltration of inflammatory granulocytes as eosinophils (Corrigan, 2008). The observed ability of FO to prevent many of these changes alone or combined with half the dose of DEX may help in management of asthma and in reducing the used dose of steroids in this respect.

In summary, since long-term use of steroids in asthma should be avoided to minimize associated adverse effects, it may be possible to use FO alone or combined with lower doses of steroids as a safe and effective mean of asthma management.

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