Effect of Thymoquinone on the Histopathological Changes of Lung and Liver in Aspergillus fumigatus Sensitized Albino Rats

El-Feki, M.A, Amin, H.M, Refat, I.H., Safi, H.M

Department of Zoology, Faculty of Science, University of Minia, Minia, Egypt

Received: 10 February 2016 / Accepted: 15 March 2016 / Publication date: 30 March 2016

ABSTRACT

The seeds of Nigella sativa are the source of Thymoquinone (TQ) which is the active ingredient of this plant. The seeds of N. sativa known as black seeds have long been used in folk medicine for a wide range of illnesses, including bronchial asthma due to the histopathological effects of TQ such as, hepatoprotective effects. The present study aims to investigate the possible modulatory effect of TQ on the histological changes of lung and liver in rats that sensitized by Aspergillus fumigatus spores (AFs). It was found that administration of TQ either before, after or with AFs enhanced the architecture, configuration, deposition of collagen fibers and distribution of mast cells in lung and liver tissues. Considering the histopathological and hepatoprotective effects of TQ reported in this study, one can conclude that TQ is effective in improvement the established histological changes of lung and liver in AF-sensitized rats.

Key words: Thymoquinone, Aspergillus fumigatus, bronchial asthma, histopathological changes, lung, liver, collagen fibers and mast cells.

Introduction

Asthma is a common immune-mediated disorder characterized by reversible airway inflammation and variable airflow obstruction with airways hyperresponsiveness (AHR). In most cases, the airway inflammation characteristic of asthma is thought to result from an allergic-type reaction to an inhaled substance from the environment (allergic asthma) (Afshar et al., 2008). Allergic asthma is a classic T helper type 2 (Th2) respiratory diseases, leading to bronchial asthma which is characterized by recurrent attacks of breathlessness and wheezing (Galli et al., 2008). In allergic asthma, allergen exposure stimulates eosinophilic inflammation of the airways associated with infiltration of T cells. Although the recruitment of eosinophils into the airways is an important component in the pathogenesis of asthma, the trafficking of T lymphocytes into the airways is now believed to establish and orchestrate the asthmatic inflammatory response (Afshar et al., 2008). In an asthma attack there were cells in the bronchial wall, called mast cells, release certain substances that cause the bronchial muscle to contract.

These substances, which include histamine and a group of chemicals called leukotrienes, also bring white blood cells into the area, which is a key part of the inflammatory response (Faoud and Ishmael, 2011). Also, liver responds to injury in a number of ways and a number of patterns have been identified like areas of abnormal tissue predominantly contain diffuse sinusoidal and portal mononuclear infiltrates (lymphocytes, Kupffer cells), vacuolated hepatocytes and aggregates of lymphocytes in portal zones may occur (Gimson, 1996). Histopathological studies in asthmatic patients have established that asthma is a process involving both central and peripheral airways. This process includes cellular changes, i.e. infiltration of the airway wall by inflammatory cells, and structural changes, i.e. thickening of all components of the airway wall (Saetta and Turato, 2001). Also, Asthma is a disorder involving all bronchial structures and depends on a complex interaction between the respiratory tract and inflammatory cells, mediators and adhesion molecules. Release of mediators primes both activation and migration of inflammatory cells that cause various degrees of airway obstruction, alternations in the mucusiliary system and hyperreactivity of the bronchial smooth muscles. Avoiding or at least minimizing exposure to asthma triggers is the most effective way of treating asthma, so it is helpful to identify which specific allergen or irritant is causing symptoms in a particular individual. Once asthma is present, symptoms may be triggered or aggravated if the individual also has rhinitis (inflammation of the lining of the nose) or sinusitis (sinus inflammation) (Allen et al., 2004).

Among the environmental triggers is the exposure to common aeroallergens, especially perennial inhalable allergens such as fungal spores that are associated with a significantly increased risk for asthma (Platts-Mills et al., 2001 & Becker and Chan-Yeung, 2008). Fungal spores especially Aspergillus fumigatus spores (AFs) are important in bronchial asthma incidence, since their growth is a common problem in moisture and water-
damaged buildings (Becker, 1994; Gravesen et al., 1994 & Becker and Chan-Yeung, 2008). Exposure to AFs on inhalation triggers an IgE-mediated allergic inflammatory response in the bronchial airways, leading to allergic bronchopulmonary aspergillosis and asthma (Sheehan and Hrachchak, 1980). Allergic responses to AFs act as oxidative stress upon cells (Goldberg and Spooner, 1983). To maintain a steady state, airway epithelial cells possess mechanisms that eliminate oxygen radicals, tending to counteract intracellular shifts toward oxygen radicals (Nakamura et al., 1997). In healthy individuals, anatomical barriers and the components of the immune system including phagocytes and cell-mediated immunity generally provide protection against infection (Maertens et al., 2001).

Oxidative damage plays an important role in the development of bronchial asthma. Increase production of reactive oxygen species leads to mutagenic alternations resulting in many histopathological processes such as, pulmonary fibrosis in rodents (Yoshimi et al., 2008) and can be implicated in pathogenesis of asthma. The imbalances between oxidants and antioxidants are believed to play a fundamental role in the pathogenesis of asthma. One key component of the oxidant-antioxidant hypothesis centers on the huge burden of oxidants derived from inflammatory cell infiltration into the lung (MacPherson et al., 2011). Among many unknown and complicated mechanisms, involvement of airways inflammation with an oxidant/antioxidant imbalance such as reactive oxygen species (ROS) can lead to lung injury as a result of direct oxidative damage to epithelial cells and cells shedding. As inflammation is often associated with an increased generation of ROS, it is rational to surmise that an oxidant stress could be mechanistically important in asthma. ROS have been shown to be associated with the pathogenesis of asthma by inducing bronchial hyperreactivity as well as directly stimulating histamine release from mast cells (Ryszard, 2000). Antioxidants from plants are reported to provide substantial protective effect that slows down the process of oxidative damage caused by ROS (Jacob and Burri, 1996). Among the promising medicinal plants, Nigella sativa, which is belonging to the Ranunculacease family. The seeds of N. sativa are the source of Thymoquinone (TQ) which is the active ingredient of this plant (Saleem, 2005). The seeds of N. sativa known as black seeds have long been used in folk medicine for a wide range of illnesses, including bronchial asthma (Al-Rowais, 2002). TQ has been shown to possess wide spectrum of activities as immunomodulatory, analgesic, antimicrobial, anti-inflammatory, bronchodilator, hepatoprotective and antioxidant properties. It was found that TQ has histopathological effects in an allergic rhinitis model as TQ reduced allergic inflammation and may be valuable for treating allergic rhinitis (Yurttaqs et al., 2016). Therefore, the histological protective effect of TQ on lung and liver inflammation of sensitized rats was examined in the present study.

Materials and Methods

Experimental animals:

One hundred and ninety eight adult male albino rats (Rattus norvegicus) weighing 100-120 g were obtained from the General Organization of Serum and Vaccine (GOSV), Helwan farm, Egypt. All animal procedures were performed in accordance to the guidelines for the care and use of experimental animals of the Committee for the Purpose of Supervision of Experiments on Animals (CPSEA) and the National Institutes of Health (NIH). The study protocol was approved by the Animal Ethics Committee of Zoology Department at the College of Science, Minia University according to Helsinki principles. The animals were allowed to acclimatize in metal cages inside a well ventilated room for 2 weeks prior to the experiment. They were maintained under standard laboratory conditions (25°C, relative humidity 60-70% and a 12 hr light/dark cycle) and were fed a diet of commercial pellets and water.

Preparation of thymoquinone:

TQ and TWEEN 20% were purchased from Sigma Chemical Company (St Louis, MO, USA). The compound was > 99% pure and reconstituted in TWEEN 20% at a concentration of 4 mg/ml. This stock was stored at 4°C in 15-ml centrifuge tubes wrapped in aluminum foil to prevent dimer formation.

Preparation of Aspergillus fumigatus spores suspension:

Aspergillus fumigatus was grown on 23 mL potato dextrose agar medium (Difco Laboratories, Detroit, MI) supplemented with 1 g L⁻¹ yeast extract (PDAY; Technical; Difco) in petri dishes (polystyrene, 100 x 15 mm) in the dark at 28°C for 5 days. Spores were carefully scraped from the agar surface; suspended in 250 mL Tween 80 (Sigma-Aldrich Chemie, St. Louis, MO) solution (0.01% vol/vol); filtered through gauze; centrifuged (20 min at 5000 g), the conidial pellet resuspended in phosphate-buffered saline (PBS; 10 mM potassium phosphate, 150 mMNaCl, pH 7.4); and the concentration of spores in the suspension was counted using a
Table 1: Configuration for the plan of the experiment

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time/weeks</th>
<th>W1</th>
<th>W2</th>
<th>W3</th>
<th>W4</th>
<th>W5</th>
<th>W6</th>
</tr>
</thead>
<tbody>
<tr>
<td>N0</td>
<td>F-I</td>
<td>F-I</td>
<td>F-I</td>
<td>F-I</td>
<td>F-I</td>
<td>F-I</td>
<td>F-I</td>
</tr>
<tr>
<td>C1 (PBS)</td>
<td>PBS</td>
<td>PBS</td>
<td>PBS</td>
<td>PBS</td>
<td>PBS</td>
<td>PBS</td>
<td>PBS</td>
</tr>
<tr>
<td>C2 (PBS+TW20%)</td>
<td>TW20(TQ)</td>
<td>PW20</td>
<td>PW20</td>
<td>PW20</td>
<td>PW20</td>
<td>PW20</td>
<td>PW20</td>
</tr>
<tr>
<td>C3 (PBS+TW20%)</td>
<td>PW20(TQ)</td>
<td>TW20</td>
<td>TW20</td>
<td>TW20</td>
<td>TW20</td>
<td>TW20</td>
<td>TW20</td>
</tr>
<tr>
<td>C4 (PBS+TW20%)</td>
<td>TW20(TQ)</td>
<td>TW20</td>
<td>TW20</td>
<td>TW20</td>
<td>TW20</td>
<td>TW20</td>
<td>TW20</td>
</tr>
<tr>
<td>T1 (AF)</td>
<td>AF(TQ)</td>
<td>PW20</td>
<td>PW20</td>
<td>PW20</td>
<td>PW20</td>
<td>PW20</td>
<td>PW20</td>
</tr>
<tr>
<td>T2 (AF-TQ)</td>
<td>AF(TQ)</td>
<td>TW20</td>
<td>TW20</td>
<td>TW20</td>
<td>TW20</td>
<td>TW20</td>
<td>TW20</td>
</tr>
</tbody>
</table>

Histological studies:

Lung and liver removed from rats were infused with 10% buffered formalin, fixed in formalin solution and processed. Sections were cut at 6 micron of thickness and stained with Hematoxylin and Eosin (H&E) for morphological changes (Kiennan, 2008), masson’s trichrome for collagen fibers (Bancroft and Stevens, 1982) and toluidine blue for mast cells (Sheehan and Hrapchak, 1980).

Results

Pathological changes in lung:

1- Hematoxylin and eosin (H&E) stain:

a- normal group:

The basic structure of normal lung sections stained with Hematoxylin and Eosin showed normal lung architecture with thin inter-alveolar septa, normal clear alveoli and Bronchi (Fig. A1&A2).
b- Control groups:

Lung sections of animals given 0.05 µ g / kg b.wt. of Phosphate Buffer Saline (PBS) alone for 6 weeks showed normal thickness of wall of alveoli and normal bronchus (Fig. A3 & A4). While, when PBS (0.05 µ g / kg b.wt.) either given for 4 weeks before TWEEN 20% (0.1 µ g / kg b.wt.) which given for 2 weeks later or given with TWEEN 20% (0.05 µ g / kg b.wt.) for 6 weeks at the same time, there were severe thickness of wall of alveoli, haemolysed blood and rupture of epithelium of bronchus (Fig. A9, A10, A7 & A8). Also, Lung sections of animals given 0.1 µ g / kg b.wt. of TWEEN 20% alone for 6 weeks showed severe thickness of wall of alveoli, proliferation of parenchyma cells, interstitial hemorrhage and inflammation between alveoli (Fig. A5 & A6). While, when TWEEN 20% (0.1 µ g / kg b.wt.) given for 2 weeks before PBS (0.05 µ g / kg b.wt.) which given for 4 weeks later, there were nearly moderate thickness of wall of alveoli with rupture in some areas of alveoli and of epithelium of bronchus (Fig. A11 & A12).

c- Treated groups:

Lung sections of animals given 0.05 µ g / kg b.wt. of Aspergillus fumigatus spores (AFs) suspension alone for 6 weeks showed severe thickness of wall of alveoli, very highly proliferation of parenchyma cells (metaplasia), necrosis, haemolysed blood, melano-macrophage centres (MMCs) and tissue destruction of alveoli and bronchus (Fig. A13, A14, A15 & A16). On the other hand, lung sections of animals treated with 0.1 µ g / kg b.wt. of thymoquinone (TQ) alone for 6 weeks showed normal thickness of wall of alveoli, normal bronchus with regular wall and there was no haemolysed blood or inflammation of lung tissue (Fig. A17 & A18). Also, injection of TQ (0.1 µ g / kg b.wt.) either for 2 weeks after AFs suspension (0.05 µ g / kg b.wt.) which given for 4 weeks or for 2 weeks before AFs suspension (0.05 µ g / kg b.wt.) which given for 4 weeks later or injection of TQ (0.05 µ g / kg b.wt.) with AFs suspension (0.05 µ g / kg b.wt.) for 6 weeks at the same time improved the pathological changes which noticed in the AFs rats whereas the thickness of wall of alveoli were reduced, bronchus was improved with regular wall except there were few interstitial hemorrhage between alveoli (Fig. A21, A22, A23, A24 & A19 & A20).

2- Masson's trichrome stain:

a- Normal group:

Normal lung sections stained with Masson's Trichrome showed normal collagen deposition around bronchi contain a thin layer of collagen (Fig. B1).

b- Control groups:

Lung sections of animals given 0.05 µ g / kg b.wt. of Phosphate Buffer Saline (PBS) alone for 6 weeks showed few collagen deposition around bronchi with clear configuration and there were no fibrosis or oedema (Fig. B2). Also, when PBS (0.05 µ g / kg b.wt.) either given for 4 weeks before TWEEN 20% (0.1 µ g / kg b.wt.) which given for 2 weeks later or with TWEEN 20% (0.05 µ g / kg b.wt.) for 6 weeks at the same time, there were few collagen deposition around bronchi (Fig. B5 & B4). While, Lung sections of animals given 0.1 µ g / kg b.wt. of TWEEN 20% alone for 6 weeks showed severe collagen deposition around bronchi with fibrosis (Fig. B3). Moreover, when TWEEN 20% (0.1 µ g / kg b.wt.) given for 2 weeks before PBS (0.05 µ g / kg b.wt.) which given for 4 weeks later, there were moderate collagen deposition around bronchi with unclear configuration, fibrosis and edema (Fig. B6)

c- Treated groups:

Lung sections of animals given 0.05 µ g / kg b.wt. of Aspergillus fumigatus spores (AFs) suspension alone for 6 weeks showed severe collagen deposition around bronchi with unclear configuration, fibrosis, edema around bronchi and hemorrhage into bronchi (moderate arrow). These all changes together were called telangiectasis (Fig. B7). On the other hand, lung sections of animals treated with 0.1 µ g / kg b.wt. of thymoquinone (TQ) alone for 6 weeks showed moderate collagen deposition around bronchi and there were no fibrosis or oedema (Fig. B8). Also, demonstration of TQ (0.1 µ g / kg b.wt.) either for 2 weeks after AFs suspension (0.05 µ g / kg b.wt.) for 4 weeks or demonstration of TQ (0.05 µ g / kg b.wt.) with AFs suspension (0.05 µ g / kg b.wt.) for 6 weeks at the same time, improved the pathological changes which noticed in the AFs rats whereas the collagen deposition around bronchi became moderate or few with clear configuration, reduced fibrosis and there was no oedema (Fig. B10 & Fig. B9). Moreover, when TQ (0.1 µ g / kg b.wt.) given for 2
weeks before AFs suspension (0.05 µ g/kg b.wt.) which given for 4 weeks later, there were moderate collagen deposition around bronchi with clear configuration and few oedema (Fig.B11).

3- Toluidine blue stain:

a- Normal group:

In normal lung sections stained with toluidine blue there was normal mast cell with clear configuration (Fig.C1).

b- Control groups:

Lung sections of animals given 0.05 µ g/kg b.wt. of Phosphate Buffer Saline (PBS) alone for 6 weeks showed nearly normal mast cell with clear configuration (Fig.C2). While, lung sections of animals given 0.1 µ g/kg b.wt. of TWEEN 20% alone for 6 weeks showed many granulated mast cells (Fig.C3). Finally, lung sections of animals given 0.05 µ g/kg b.wt. of TWEEN 20% with 0.05 µ g/kg b.wt. of PBS for 6 weeks at the same time showed, accumulation of degranulated mast cells around portal tract with clear configuration (Fig.C4).

C- treated groups:

Lung sections of animals given 0.05 µ g/kg b.wt. of Aspergillus fumigatus spores (AFs) suspension alone for 6 weeks showed granulated mast cell with clear configuration (Fig.C7). On the other hand, lung sections of animals treated with 0.1 µ g/kg b.wt. of thymoquinone (TQ) alone for 6 weeks showed few mast cells with clear configuration (Fig.C8). Also, TQ (0.1 µ g/kg b.wt.) demonstration for 2 weeks after AFs suspension (0.05 µ g/kg b.wt.) which given for 4 weeks improved the pathological changes which noticed in the AFs rats whereas there was normal mast cell with clear configuration (Fig.C10). While, when TQ (0.1 µ g/kg b.wt.) either given for 2 weeks before AFs suspension (0.05 µ g/kg b.wt.) which given for 4 weeks later or TQ (0.05 µ g/kg b.wt.) given with AFs suspension (0.05 µ g/kg b.wt.) for 6 weeks at the same time, there were degranulated mast cells (Fig.C9 & C11).

Pathological changes in liver:

1- Hematoxylin and eosin (H&E) stain:

a- Normal group:

The basic structure of normal liver sections showed numerous hepatic lobules. Each lobule consists of a central vein surrounded by tiny liver cells grouped in strands. These cells perform the work of the liver and known as hepatocytes. Cavities known as sinusoids separate the groups of cells within a lobule. The hepatocytes are polyhedral with granulated eosinophilic cytoplasm and centrally located nuclei with one or two nucleoli and granules of chromatin. Portal tract is a distinctive arrangement in the liver and it is a component of the hepatic lobule. Also, kupffer cells appeared between hepatic cells as spindle-shaped cells (Fig.D1&D2).

b- Control groups:

Liver sections of animals given 0.05 µ g/kg b.wt. of Phosphate Buffer Saline (PBS) alone for 6 weeks showed regular wall of central vein and portal tract and hepatocytes were arranged in regular strands with narrow hepatic sinusoids (Fig.D3&D4). Also, liver sections of animals given 0.05 µ g/kg b.wt. of PBS either with 0.05 µ g/kg b.wt. of TWEEN 20% for 6 weeks at the same time or for 4 weeks after 0.1 µ g/kg b.wt. of TWEEN 20% which given for 2 weeks showed arranged wall of central vein and portal tract, arranged hepatocyte strands and activated kupffer cells (Fig.D7, D8, D11&D12). While, liver sections of animals given 0.1 µ g/kg b.wt. of TWEEN 20% either alone for 6 weeks or for 2 weeks after PBS (0.05 µ g/kg b.wt.) which given for 4 weeks, showed rupture wall of central vein and portal tract. Also, central vein was congested with haemolysed blood and portal tract was infiltrated with leukocytes. Moreover, hepatocytes lost their stranded pattern of arrangement with vacuolation in cytoplasm (Fig.D5, D6, D9&D10).
Fig. A: Intranasal administration of AFs induces marked changes in the histological architecture of the lung that is reversed by TQ treatment. Sections of normal rat lung showing normal thickness of wall of alveoli (alv.) (thin arrow) (A1) and normal bronchus (Br.) (A2). Lung section of rat at 6th week post-injection of 0.05 µg/kg b.wt. of PBS showing nearly normal thickness of wall of alveoli (thin arrow) (A3) and normal bronchus (moderate arrow) (A4). Lung section of rat at 6th week post-injection of 0.1 µg/kg b.wt. of TWEEN 20% showing haemolysed blood, inflammation in the wall of alveoli, into the bronchus (moderate arrows) and severe thickness of wall of alveoli (thin arrows) (A5&A6). Lung section of rat at 6th week post-injection of 0.05 µg/kg b.wt. of TWEEN 20% followed by 0.05 µg/kg b.wt. of PBS, showing nearly moderate thickness of wall of alveoli (thin arrows) with rupture in some areas of alveoli and of epithelium of bronchus (stars) (A11&A12). Lung section of rat at 6th week post-injection of 0.05 µg/kg b.wt. of AFs followed by 0.1 µg/kg b.wt. of TQ showing moderate thickness of wall of alveoli (thin arrow) (A19) and nearly moderate bronchus (A20). Lung section of rat at 6th week post-injection of 0.05 µg/kg b.wt. of TQ followed by 0.05 µg/kg b.wt. of AFs showing nearly moderate thickness of wall of alveoli (thin arrows) (A23), nearly moderate bronchus with fewer haemolysed blood (moderate arrow) and infiltration of red blood cells into the bronchus (thick arrow) (A24) (H&E, X 400).
Fig. B: Intranasal administration of AFs induces marked changes in the collagen deposition in the lung that is reversed by TQ treatment. Sections of normal rat lung showing normal collagen deposition around bronchus (Br.) with clear configuration (thick arrow) (B1). Lung section of rat at 6th week post-injection of 0.05 µ g/kg b.wt. of PBS showing nearly normal collagen deposition around the bronchus with clear configuration (thick arrow) (B2). Lung section of rat at 6th week post-injection of 0.1 µ g/kg b.wt. of TWEEN 20% showing severe collagen deposition around bronchus (thick arrow) with fibrosis (thin arrow) (B3). Lung section of rat at 6th week post-injection of 0.05 µ g/kg b.wt. of PBS with 0.05 µ g/kg b.wt. of TWEEN 20% at the same time, showing few collagen deposition around bronchus (thick arrows) (B4). Lung section of rat at 6th week post-injection of 0.05 µ g/kg b.wt. of PBS followed by 0.1 µ g/kg b.wt. of TWEEN 20%, showing few collagen deposition around bronchus (thick arrows) (B5). Lung section of rat at 6th week post-injection of 0.1 µ g/kg b.wt. of TWEEN 20% followed by 0.05 µ g/kg b.wt. of PBS, showing moderate collagen deposition around bronchus with unclear configuration (thick arrow), fibrosis (thin arrow) and edema (moderate arrow) (B6). Lung section of rat at 6th week post-injection of 0.05 µ g/kg b.wt. of AFs, showing severe collagen deposition with unclear configuration around bronchus (thick arrow), fibrosis (star), edema (thin arrow) and hemorrhage into bronchus (moderate arrow). These all changes together were called telangiectasis (B7). Lung section of rat at 6th week post-injection of 0.1 µ g/kg b.wt. of TQ, showing moderate collagen deposition with clear configuration around bronchus (thick arrow) and there were no fibrosis or edema (B8). Lung section of rat at 6th week post-injection of 0.05 µ g/kg b.wt. of AFs with 0.05 µ g/kg b.wt. of TQ at the same time, showing few collagen deposition around bronchus (thick arrow) and few fibrosis (thin arrow) (B9). Lung section of rat at 6th week post-injection of 0.05 µ g/kg b.wt. of AFs followed by 0.1 µ g/kg b.wt. of TQ, showing moderate collagen deposition around bronchus (thick arrow) and few fibrosis (thin arrow) (B10). Lung section of rat at 6th week post-injection of 0.1 µ g/kg b.wt. of TQ followed by 0.05 µ g/kg b.wt. of AFs, showing moderate collagen deposition with clear configuration around bronchus (thick arrow) and few oedema (thin arrow) (B11) (M.T., X 400).
Fig. C: Intranasal administration of AFs induces marked changes in the amount and configuration of mast cells in the lung tissue that is reversed by TQ treatment. Sections of normal rat lung showing normal mast cell with clear configuration (thin arrow) (C1). Lung section of rat at 6th week post-injection of 0.05 µg/kg b.wt. of PBS showing nearly normal mast cell with clear configuration (thin arrow) (C2). Lung section of rat at 6th week post-injection of 0.1 µg/kg b.wt. of TWEEN 20% showing many granulated mast cells with clear configuration (thin arrows) (C3). Lung section of rat at 6th week post-injection of 0.05 µg/kg b.wt. of PBS with 0.05 µg/kg b.wt. of TWEEN 20% at the same time, showing accumulation of degranulated mast cells with clear configuration (thin arrows) (C4). Lung section of rat at 6th week post-injection of 0.05 µg/kg b.wt. of PBS followed by 0.1 µg/kg b.wt. of TWEEN 20%, showing fewer mast cell with unclear configuration (thin arrow) (C5). Lung section of rat at 6th week post-injection of 0.1 µg/kg b.wt. of TWEEN 20% followed by 0.05 µg/kg b.wt. of PBS, showing fewer mast cell with unclear configuration (thin arrow) (C6). Lung section of rat at 6th week post-injection of 0.05 µg/kg b.wt. of AFs, showing fewer granulated mast cell with clear configuration (thin arrow) (C7). Lung section of rat at 6th week post-injection of 0.1 µg/kg b.wt. of TQ, showing many mast cells with clear configuration (thin arrows) (C8). Lung section of rat at 6th week post-injection of 0.05 µg/kg b.wt. of AFs with 0.05 µg/kg b.wt. of TQ at the same time, showing degranulated mast cell with clear configuration (thin arrow) (C9). Lung section of rat at 6th week post-injection of 0.05 µg/kg b.wt. of AFs followed by 0.1 µg/kg b.wt. of TQ, showing mast cell with clear configuration (thin arrow) (C10). Lung section of rat at 6th week post-injection of 0.1 µg/kg b.wt. of TQ followed by 0.05 µg/kg b.wt. of AFs, showing degranulated mast cell (thin arrow) (C11) (T.b. X 1000).

c- Treated groups:

Liver sections of animals given 0.05 µg/kg b.wt. of Aspergillus fumigatus spores (AFs) suspension alone for 6 weeks showed congestion of portal tract and central vein with leukocytes and haemolysed blood, activated kupffer cells in sinusoids and hepatocytes lost their stranded pattern of arrangement with vacuolation in cytoplasm (Fig.D13&D14). On the other hand, liver sections of animals treated with 0.1 µg/kg b.wt. of thymoquinone (TQ) alone for 6 weeks showed regular wall of portal tract and central vein and hepatocytes were
arranged in regular strands with moderate hepatic sinusoids (Fig.D15&D16). Signs of histological improvement could be observed in rats treated with TQ (0.1 µg/kg b.wt.) for 2 weeks post AFs suspension (0.05 µg/kg b.wt.) for 4 weeks, as good architecture of liver tissue, arrangement of hepatocytes in strands with widened hepatic sinusoids and regular wall of central vein and portal tract except there were activated kupffer cells in hepatic sinusoids (Fig.D19&D20). Also, administration of TQ (0.05 µg/kg b.wt.) either with AFs suspension (0.05 µg/kg b.wt.) for 6 weeks at the same time or administration of TQ (0.1 µg/kg b.wt.) for 2 weeks before AFs suspension (0.05 µg/kg b.wt.) which given for 4 weeks later led to arrangement of hepatocytes in strands in some areas and nearly arranged wall of central vein and portal tract except there were destruction of tissue in other areas, fewer haemolysed blood around portal tract and activated kupffer cells between strands (Fig.D17, D18, D21&D22).

2- Masson's trichrome stain:

a- Normal group:

Normal liver sections stained with Masson's Trichrome showed normal collagen deposition around portal tract and central vein with clear configuration (Fig.E1&E2).

b- Control groups:

Liver sections of animals given 0.05 µg/kg b.wt. of Phosphate Buffer Saline (PBS) alone for 6 weeks showed few collagen deposition around the central vein and portal tract with clear configuration (Fig.E3&E4). Also, when PBS (0.05 µg/kg b.wt.) given either for 4 weeks before TWEEN 20% (0.1 µg/kg b.wt.) which given for 2 weeks later or with TWEEN 20% (0.05 µg/kg b.wt.) for 6 weeks at the same time, there were few collagen deposition around central vein and portal tract except the unclear configuration of collagen fibers. (Fig.E7, E8, E9&E10). On the other hand, liver sections of animals given 0.1 µg/kg b.wt. of TWEEN 20% alone for 6 weeks, showed severe collagen deposition around central vein with hyaline configuration and severe collagen deposition around portal tract with fibrosis and edema (Fig. E5&E6). Finally, liver sections of animals given 0.1 µg/kg b.wt. of TWEEN 20% for 2 weeks before PBS (0.05 µg/kg b.wt.) which given for 4 weeks later, showed moderate collagen deposition around central vein and portal tract with hyaline configuration (Fig.E11&E12).

c- Treated groups:

Liver sections of animals given 0.05 µg/kg b.wt. of Aspergillus fumigatus spores (AFs) suspension alone for 6 weeks showed severe collagen deposition around portal tract and central vein with hyaline configuration and there were fibrosis and edema around portal tract (Fig.E13&E14). On the other hand, liver sections of animals treated with 0.1 µg/kg b.wt. of thymoquinone (TQ) alone for 6 weeks showed few collagen deposition around portal tract and central vein with nearly clear configuration and there was no fibrosis or edema (Fig.E15&E16). Also, TQ (0.1 µg/kg b.wt.) demonstration for 2 weeks after AFs suspension (0.05 µg/kg b.wt.) which given for 4 weeks improved the pathological changes which noticed in the AFs rats whereas the collagen deposition around portal tract and central vein became moderate with clear configuration and there was no fibrosis or edema (Fig.E19&E20). Finally, liver sections of animals given 0.05 µg/kg b.wt. of TQ either with 0.05 µg/kg b.wt. of PBS for 6 weeks at the same time or 0.1 µg/kg b.wt. of TQ for 2 weeks before 0.05 µg/kg b.wt. of AFs suspension which given for 4 weeks later, showed few collagen deposition around central vein and portal tract with few fibrosis or edema around portal tract (Fig.E17, E18, E21&E22).

3- Toluidine blue stain:

a- Normal group:

In normal liver sections stained with Toluidine Blue there were few mast cells can be detected around portal tract with normal and clear configuration (Fig.F1).

b- Control group:

Liver sections of animals given 0.05 µg/kg b.wt. of Phosphate Buffer Saline (PBS) either alone for 6 weeks or given PBS (0.05 µg/kg b.wt.) for 4 weeks before TWEEN 20% (0.1 µg/kg b.wt.) which given for 2 weeks later, showed few mast cells around portal tract with clear configuration (Fig.F2 & F5). While, liver sections of animals given 0.1 µg/kg b.wt. of TWEEN 20% alone for 6 weeks showed accumulation of...
degranulated mast cells around portal tract with unclear configuration (Fig. F3). Also, liver sections of animals given 0.05 µ g/kg b.wt. of TWEEN 20% with 0.05 µ g/kg b.wt. of PBS for 6 weeks at the same time, showed accumulation of degranulated mast cells around portal tract but with clear configuration (Fig. F4). Finally, liver sections of animals given 0.1 µ g/kg b.wt. of TWEEN 20% for 2 weeks before 0.05 µ g/kg b.wt. of PBS which given for 4 weeks later, showed rare number of mast cells around portal tract with clear configuration (Fig. F6).

c- Treated groups:

Liver sections of animals given 0.05 µ g/kg b.wt. of Aspergillus fumigatus spores (AFs) suspension alone for 6 weeks showed accumulation of degranulated mast cells around portal tract with clear configuration (Fig. F7). On the other hand, liver sections of animals given 0.1 µ g/kg b.wt. of thymoquinone (TQ) either alone for 6 weeks or given TQ (0.1 µ g/kg b.wt.) for 2 weeks after AFs suspension (0.05 µ g/kg b.wt.) for 4 weeks or before AFs suspension (0.05 µ g/kg b.wt.) which given for 4 weeks later, showed marked depletion of numbers of mast cells around portal tract with clear configuration (Fig. F8, F10 & F11). Finally, liver sections of animals given 0.05 µ g/kg b.wt. of TQ with 0.05 µ g/kg b.wt. of AFs suspension for 6 weeks at the same time, showed many mast cells with unclear configuration (Fig. F9).
Fig. D: Intranasal administration of AFs induces marked changes in the histological architecture of the liver that is reversed by TQ treatment. Sections of normal rat liver showing normal hepatocytes (thin arrows), central vein (C.V.), sinusoids (thick arrows), kupffer cells (moderate arrows) and portal tract (P.T.) (D1&D2). Liver section of rat at 6th week post-injection of 0.05 µg/kg b.wt. of PBS showing regular hepatocyte strands with normal sinusoids (thin arrows), arranged wall of central vein (thick arrow) (D3) and portal tract (P.T.) (thick arrow) (D4). Liver section of rat at 6th week post-injection of 0.1 µg/kg b.wt. of TWEEN 20% showing distribution of hepatocyte strands, vaculation of cytoplasm (thin arrows), interrupted wall of central vein and portal tract (moderate arrows) (D5 & D6). Liver section of rat at 6th week post-injection of 0.05 µg/kg b.wt. of PBS with 0.05 µg/kg b.wt. of TWEEN 20% at the same time, showing arranged wall of central vein (thick arrow) (D7), arranged hepatocyte strands in some areas (thin arrows) and activated kupffer cells (moderate arrows) (D7 & D8). Liver section of rat at 6th week post-injection of 0.05 µg/kg b.wt. of PBS followed by 0.1 µg/kg b.wt. of TWEEN 20%, showing distribution of hepatocyte strands, vaculation of cytoplasm (thin arrows) (D9 & D10), interrupted wall of central vein (moderate arrows), central vein was congested with haemolysed blood (thick arrow) (D9) and portal tract was infiltrated with lymphocytes (thick arrow) (D10). Liver section of rat at 6th week post-injection of 0.1 µg/kg b.wt. of TWEEN 20% followed by 0.05 µg/kg b.wt. of PBS, showing arranged wall of central vein and portal tract (thick arrows), arranged hepatocyte strands (thin arrows) and activated kupffer cells (moderate arrows) (D11 & D12). Liver section of rat at 6th week post-injection of 0.05 µg/kg b.wt. of AFs, showing distribution of hepatocyte strands, vaculation of cytoplasm (moderate arrows), portal tract and central vein were infiltrated with lymphocytes (thick arrows) and haemolysed blood (wavy line) and there were activated kupffer cells in sinusoids (thin
arrows) (D13 & D14). Liver section of rat at 6th week post-injection of 0.1 µ g/kg b.wt. of TQ, showing arranged hepatocyte strands with wide sinusoids (thin arrows), nearly arranged wall of central vein (moderate arrow) (D15) and nearly arranged wall of portal tract (thick arrow) (D16). Liver section of rat at 6th week post-injection of 0.05 µ g/kg b.wt. of AFs with 0.05 µ g/kg b.wt. of TQ at the same time, showing nearly arranged hepatocyte strands in some areas (thin arrows), nearly arranged wall of central vein and portal tract (moderate arrows), destruction of tissue in some areas (stars) (D17 & D18) and there was few haemolysed blood around portal tract (thick arrow) (D18). Liver section of rat at 6th week post-injection of 0.05 µ g/kg b.wt. of AFs followed by 0.1 µ g/kg b.wt. of TQ, showing nearly arranged hepatocyte strands in some areas with widened sinusoids (thin arrows), nearly arranged wall of central vein and portal tract (thick arrow) and there were activated kupffer cells between strands (moderate arrows) (D19 & D20). Liver section of rat at 6th week post-injection of 0.1 µ g/kg b.wt. of TQ followed by 0.05 µ g/kg b.wt. of AFs, showing nearly arranged hepatocyte strands in some areas (thin arrows), destruction of tissue in other areas (stars) and activated kupffer cells between strands (moderate arrows) (D21 & D22) (H&E, X 400).
Fig. E: Intranasal administration of AFs induces marked changes in the collagen deposition in the liver that is reversed by TQ treatment. Sections of normal rat liver showing normal collagen deposition around central vein (C.V.) and portal tract (P.T.) with clear configuration (thick arrows) (E1&E2). Liver section of rat at 6th week post-injection of 0.05 µg/kg b.wt. of PBS showing moderate collagen deposition around the central vein and portal tract with clear configuration (thick arrows) (E3&E4). Liver section of rat at 6th week post-injection of 0.1 µg/kg b.wt. of TWEEN 20% showing severe collagen deposition around central vein with hyaline configuration (thick arrow) (E5), there were fibrosis (thin arrow) and edema (moderate arrow) around portal tract (E6). Liver section of rat at 6th week post-injection of 0.05 µg/kg b.wt. of PBS with 0.05 µg/kg b.wt. of TWEEN 20% at the same time, showing few collagen deposition around central vein and portal tract with unclear configuration (thick arrows) (E7&E8). Liver section of rat at 6th week post-injection of 0.05 µg/kg b.wt. of PBS followed by 0.1 µg/kg b.wt. of TWEEN 20%, showing few collagen deposition around central vein and portal tract with unclear configuration (thick arrows) (E9&E10). Liver section of rat at 6th week post-injection of 0.1 µg/kg b.wt. of TWEEN 20% followed by 0.05 µg/kg b.wt. of PBS, showing moderate collagen deposition around central vein and portal tract with unclear configuration (thick arrows) (E11&E12). Liver section of rat at 6th week post-injection of 0.05 µg/kg b.wt. of AFs, showing severe collagen deposition around central vein and portal tract with hyaline configuration (thick arrows) (E13 & E14), there were Fibrosis (star) and edema (thin arrow) around portal tract (E14). Liver section of rat at 6th week post-injection of 0.1 µg/kg b.wt. of TQ, showing fewer collagen deposition around central vein and portal tract (thick arrows), there were no fibrosis (E15&E16). Liver section of rat at 6th week post-
Intranasal administration of AFs induces marked changes in the amount and configuration of mast cells in the liver tissue that is reversed by TQ treatment. Sections of normal rat liver showing normal mast cells around portal tract (P.T.) with normal and clear configuration (thin arrows) (F1). Liver section of rat at 6th week post-injection of 0.05 µg/kg b.wt. of PBS showing mast cells around portal tract with nearly normal and clear configuration (thin arrow) (F2). Liver section of rat at 6th week post-injection of 0.1 µg/kg b.wt. of TWEEN 20% showing accumulation of degranulated mast cells around portal tract with unclear configuration (thick arrows) (F3). Liver section of rat at 6th week post-injection of 0.05 µg/kg b.wt. of PBS followed by 0.1 µg/kg b.wt. of TWEEN 20% at the same time, showing accumulation of degranulated mast cells around portal tract with clear configuration (thin arrows) (F4). Liver section of rat at 6th week post-injection of 0.05 µg/kg b.wt. of PBS followed by 0.05 µg/kg b.wt. of AFs showing fewer mast cells around portal tract whereas some of them with clear nucleus (thin arrow) and the others with unclear configuration (thick arrow) (F5). Liver section of rat at 6th week post-injection of 0.1 µg/kg b.wt. of TWEEN 20% followed by 0.05 µg/kg b.wt. of PBS, showing fewer mast cell around portal tract with clear configuration (thin arrow) (F6). Liver section of rat at 6th week post-injection of 0.05 µg/kg b.wt. of AFs, showing accumulation of degranulated mast cells around portal tract with clear configuration (thin arrows) (F7). Liver section of rat at 6th week post-injection of 0.1 µg/kg b.wt. of TQ, showing fewer mast cell around portal tract with clear configuration (thin arrow) (F8). Liver section of rat at 6th week post-injection of 0.05 µg/kg b.wt. of AFs with 0.05 µg/kg b.wt. of TQ at the same time, showing many mast cells around portal tract with unclear configuration.
including a subset of regulatory T cells (Branton and Kopp, 1999). In asthmatics, increases in airway TGF
beta superfamily of cytokines
Transforming growth factor beta 1 (TGF-β1) which is a polypeptide member of the transforming growth factor
beta superfamily of cytokines known to induce fibrosis, is produced by a range of airway and immune cells
including a subset of regulatory T cells (Branton and Kopp, 1999). In asthmatics, increases in airway TGF-β1
levels have also been demonstrated (Vignola et al., 1997), and TGF-β1 induces collagen synthesis in bronchial
Discussion:

The possible modulatory effect of TQ on the histological changes in lung and liver induced by exposure to
AFs was investigated.

First, lung sections of rat at 6th week post-injection of 0.05 μ g / kg b.wt. of AFs showed severe thickness
of wall of alveoli, tissue destruction (metaplasia and necrosis), haemolysed blood and melano-macrophage
centres (MMCs). Also, the liver section of the same group demonstrated disruption of hepatocyte strands,
vaculation of cytoplasm, portal tract and central vein were infiltrated with leukocytes and activated kupffer cells
in sinusoids. Furthermore, in liver and lung sections of the same group, severe collagen deposition with unclear
configuration, fibrosis. oedema and many degranulated mast cells were observed. Thickness of wall of alveoli
and severe collagen deposition with unclear configuration in liver and lung may be due to the proliferation of
smooth muscle induced by inflammatory mediators (Noveral and Grunstein, 1992), cytokines and growth
factors (Stewart et al., 1994). It is now well accepted that, in asthma, there is increased deposition of collagen
types I, III and V, as elegantly shown by Roche et al. (1989), and so is more properly called "subepithelial
fibrosis". This collagen deposition may be important, since it may contribute to the total thickness of the airway
wall (Chu et al., 1998). Also, increases in airway collagen deposition, are thought to contribute to the
development of chronic lung function impairment. Lung function impairment with airflow limitation is thought
to be the most threatening consequence of severe asthma (McMillan and Lloyd, 2004). Tissue destruction
(metaplasia and necrosis) of lung may result from fibrotic changes and airway remodelling which result from
uncontrolled airway inflammation due to asthma (Fabbri et al., 1998). This airway inflammation may result
from enhanced macrophage recruitment during asthmatic process which may result in the accumulation of tissue
macrophages, which are activated by Th2 cytokines, leading to Th2 polarized reaction and inflammation
(Mantovani et al., 2005). Thus, the recruited lung macrophages have the potential to promote asthmatic
development. Several reports suggest that macrophages are crucial for down-regulating the initiation and
progression of allergic asthma (Vissers et al., 2005). Haemolysed blood in lung tissue may be due to the airway
inflammation because in inflammation there were depletion of circulating platelets which release agents that
have the potential to decrease vascular permeability (Stokes and Granger, 2012). Melano-macrophage centres,
also known as macrophage aggregates, are distinctive groupings of pigment-containing cells within the tissues
of vertebrates. Melano-macrophage centres act as focal depositories for resistant intracellular bacteria, from
which chronic infections may develop. Antigen trapping and presentation to lymphocytes, sequestration of
products of cellular degradation and potentially toxic tissue materials, such as melamins, free radicals and
catabolic breakdown products are among functions that have been ascribed (Agius and Roberts, 2003). The
melano-macrophage centres (MMCs) that observed in lung tissue may be due to the fact that melano-
macrophage centres develop focally in association with late stages of the chronic inflammatory response to
severe tissue damage and in association with the cellular response to a variety of infections (Roberts, 1976) or
may be due to β-glucan, which activates the phagocytic cells and melanomacrophages as it is a type of
polysaccharides identified to date in the cell wall of all filamentous fungi (Fontaine et al., 1996). Infiltration
of lymphocytes in sinusoids of liver because lymphocytes are recruited to inflamed tissues via adhesion molecule
and chemokine expression when an acute inflammatory stimulus trigger increased adhesion molecule and
chemokine expression by vascular endothelial cells and adjacent tissue. Adhesion molecules act by capturing
lymphocytes from the blood stream. Chemokines facilitate transmigration of leukocytes out of the blood vessels
and to the inflamed tissue (Malik and Lo, 1996). Changes in liver after Aspergillus fumigatus sensitization may be
due to Kupffer cells which are the major producers of eicosanoids, such as prostaglandin (PG) E2, D2 and
thromboxane A2, in liver (Decker , 1990). The pattern of prostanoid release by Kupffer cells depends on the
type of their activation. Inflammatory agents, viruses or cytokines induce PGE2 (Hempel et al., 1994). Eicosanoid production by liver macrophages may contribute to liver cell damage (Hewett and Roth, 1993).
Hepatic fibrosis is a dynamic cascade followed by the activation of the inflammatory cells including
macrophage and the release of fibrogenic mediators (Friedman, 2003). Excessive deposition of extracellular
matrix components such as collagen protein is an important factor in liver fibrosis (Nyberg et al., 1988).
Fibrogenesis may occur as a consequence of an ongoing inflammatory process. Lung fibrosis may be due to
Transforming growth factor beta 1 (TGF-β1), which is a polypeptide member of the transforming growth factor
beta superfamily of cytokines known to induce fibrosis, is produced by a range of airway and immune cells
including a subset of regulatory T cells (Branton and Kopp, 1999). In asthmatics, increases in airway TGF-β1
levels have also been demonstrated (Vignola et al., 1997), and TGF-β1 induces collagen synthesis in bronchial
fibroblasts and transforms them to myofibroblasts (Richer et al., 2001). Considering these findings it seems likely that TGF-β1, induced by persistent airway inflammation, is an important profibrotic factor following chronic allergen exposure. Minimal perivascular oedema was observed in lung and liver of AFs-sensitized animals which may be due to the sensitization of mice with AFs which induced the production of Th2 cytokines including IL-4, IL-5 and IL-10. IL-4 is a key cytokine that plays a central role in the development of allergic inflammatory responses where, IL-4 modulate the CD80, CD86 and OX40L costimulatory molecules in AFs-sensitized mice (Knutsen, 2003) or may be due to the following mechanisms: pulmonary venular constriction (Eliakim and Aviado, 1961); stimulation of an ematogenic cell in the medulla of the central nervous system (Luisada, 1940); and increase in permeability of pulmonary capillaries (Bariety and Kohler, 1951) which may be due to increase in number of degranulated mast cells after exposure to AFs whereas, these degranulated mast cells release histamine, which in turn increases the permeability of capillaries. Increase of mast cell numbers in asthma may be due to the activation of mast cells by IgE receptor cross linking. In response to activation, preformed mediators that are stored bound to proteoglycans, for example, TNF-α, IL-1α, IL-13, histamine, tryptase and chymase, are released. New synthesis of arachidonic acid metabolites (leukotriene C4 (LTC4), leukotriene B4 (LTB4) and prostaglandin D2 (PGD2)) and further cytokines are stimulated. Mediators from degranulating mast cells are critical to the pathology of asthma (Hart, 2001). On the other hand, we observed in lung and liver sections of rat at 6th week post-injection of 0.1 μ g ⁄ kg b.wt. or 0.05 μ g ⁄ kg b.wt. of thymoquinone either alone, after, before or with AFs most of these histological changes were improved by TQ supplementation except there were few degranulated mast cells that noticed in administration of TQ either for 2 weeks before AFs suspension which given for 4 weeks later or with AFs suspension for 6 weeks at the same time in case of lung or when TQ was demonstrated with AFs suspension for 6 weeks at the same time in case of liver whereas, many mast cells with unclear configuration were noticed. This degranulated mast cells may be due to the exposure to AFs as mentioned before. While, the improvement by TQ injection may be due to its antioxidant effect as reported by Salem (2005), in a review article, provided clear evidence that both the oil of N. sativa and its active ingredients, in particular TQ, possess reproducible anti-oxidant effects and potent anti-inflammatory effects on several inflammation-based models by suppression of the inflammatory mediators, prostaglandins, and leukotrienes or may be due to its suppressing effects on inflammation. In fact, the inhibitory effects of the essential oil of N. sativa have been shown in both cyclooxygenase and 5-lipoxygenase pathways of arachidonic acid metabolism and also in membrane lipid peroxidation (Houghton et al., 1995; Boskabady and Farhadi, 2008). Also, the enhancement of lung tissue after treatment with TQ may be due to the immunomodulatory and cytoprotector properties of Nigella sativa oil (NSO). Treatment with NSO significantly attenuated the pulmonary lesion by maintaining better alveolarization and reducing fibrosis, inflammation and oxidative stress (Cuneyt et al., 2013). These findings together with the known histopathological effects of TQ suggest that TQ has a potential improvement effect on asthma. However, further studies with long-term treatments which evaluate the effects of TQ on lung inflammation and remodeling are needed.

References


