Original Article

Anti-asthmatic and Anti-allergic Effects of Thymoquinone on Experimentally- Induced Hypersensitivity

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A B S T R A C T

Nigella sativa L. has been used in folk medicine for treatment of many diseases. Thymoquinone (TQ), a main constituent of its oil and seeds, has shown promising medicinal properties in the treatment and prevention of various diseases. The present study aims to investigate the potential effect of TQ on airway-induced hypersensitivity. Ovalbumin (OVA) sensitization and challenge in guinea pig tracheal muscle preparation were used in order to investigate the anti-asthmatic activity of TQ. To study the effect of TQ on acute lung injury, lipopolysaccharide (LPS) - induced lung injury method was used. In addition, rat peritoneal mast cells (RPMCs) were collected to investigate the release of histamine from these cells. Furthermore, to study the anti-allergic activity of TQ, the systemic anaphylactic shock technique induced by compound 48/80 was performed.

Pretreatment with TQ (3 mg/kg, i.p.) for 5 days prior to ovalbumin sensitization showed a marked decrease in the response of the tracheal spirals to acetylcholine and histamine, as spasmogens in a cumulative dose response–curve. TQ (8mg/kg, i.p.) prevented most of the pathological detrimental changes that occurred in response to the endotoxin LPS as the inflammatory cells infiltration, lipid peroxidation (LP), glutathione depletion (GSH), tumor necrosis factor-alpha (TNF- α) and interlukin-1 beta (IL-1β) levels in both boronchoalevolar lavage fluid (BALF) and lung tissue homogenates. Sensitization of rats induced a significant increase in the histamine release from RPMCs which is inhibited by pretreatment with TQ (8 mg/kg, i.p.). Similarly, pretreatment of mice with TQ (50 and 100 mg/kg), 1hr prior to injection of compound 48/80 (8mg/kg, i.p.) significantly inhibited the % of mortality of mice following the systemic anaphylactic reaction. Considering the anti-asthmatic, anti-inflammatory, antioxidant and anti-allergic activities of TQ reported in this study, one can conclude that TQ could be of therapeutic potentials in treating various diseases associated with airway-induced hypersensitivity.

Key Words: Thymoquinone, hypersensitivity, anaphylaxis, allergy, antioxidants, trachea, cytokines.

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1. INTRODUCTION

Asthma is a common chronic disorder of the airways characterized by airflow obstruction, bronchial hyper-responsiveness, and an underlying inflammation *(Busse and Lemanske, 2001)*. Inflammation has a central role in the pathophysiology of asthma. It involves an interaction of many cell types and multiple mediators with the airways that eventually results in the characteristic features of the disease *(O'Byrne, 2009)*. Many inflammatory cells, including eosinophils, mast cells, macrophages and neutrophils, are involved in the pathogenesis of airway inflammation in asthma *(Kelly et al., 1998)*. These cells produce more reactive oxygen species (ROS) *(Cluzel et al., 1987)* that affect airway smooth muscles and simulate histamine release from mast cells *(Adler et al., 1990)*.

Airway hypersensitivity is associated with the pathophysiology of asthma, acute lung injury and anaphylaxis.

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are among the major causes of respiratory failure and are associated with a high frequency of mortality and morbidity *(Ware and Matthay, 2000)*. Lipoplysaccharide (LPS) induces intense lung inflammation, with macrophage activation and recruitment of neutrophils to the intersticium, alveoli, and to the airways of guinea-pigs *(Gordon et al., 1991)*, rats *(Ulich et al., 1994)*, and mice *(Harmsen, 1988)*. Neutrophil recruitment is accompanied by an augmented lung vascular permeability. Since these are the characteristic features of ALI/

ARDS, LPS-induced lung inflammation has been used as a model for these syndromes *(Wheeldon, et al., 1992; Ware and Matthay, 2005)*. Tumor necrosis factor-alpha (TNF- α) and interlukin-1 beta (IL-1β) have been identified as important pathogenic mediators of LPS-induced ALI. During sepsis, the alveolar compartmentalization is lost, allowing passage of cytokines, which were released into the bloodstream and to the pulmonary endothelium. These cytokines have important roles in lung dysfunction *(Simpson and Casey, 1989)*.

Anaphylaxis is an acute hypersensitivity reaction with multi-organ-system involvement that rapidly progresses to, a severe life-threatening reaction *(Madaan and Maddox, 2003)*. Mast cells are the primary effector cells involved in an allergic or immediate hypersensitivity response. Activation of mast cells occurs in response to a challenge by a specific antigen against which the surface immunoglobulin E (IgE) is directed, or by other IgE-directed ligands. Activated mast cells can produce histamine and a wide variety of inflammatory mediators *(Kalesnikoff & Galli, 2008)* which result into various acute and chronic allergic responses. Mast cell degranulation can also be elicited by the synthetic compound 48/80 *(Ennis et al., 1980)*. Compared with the natural process, compound 48/80 induces histamine release from mast cells and is so used as a direct and convenient reagent to investigate the mechanisms of allergy and anaphylaxis *(Allansmith et al., 1989)*.

Among the promising medicinal plants used nowadays to relieve the symptoms of allergy and asthma is the Nigella sativa, in which many of the reported pharmacological effects are due to thymoquinone (TQ); the major active constituent of the volatile oil *(El-Dakhakhany, 1982; Labib, 2005)*. TQ has been shown to exert anti-inflammatory effects in a number of diseases including bronchial asthma *(Kalus et al., 2003)*.

The present study was performed in order to investigate the possible anti-asthmatic and anti-allergic activities of TQ and to explore the underlying mechanisms of action against airway-induced hypersensitivity. These effects were studied in-vitro to study the effect of TQ on ovalbumin (OVA)-sensitized trachea of guinea pigs and in-vivo in three animal models namely; LPS-induced lung injury, histamine release from rat peritoneal mast cells and the systemic anaphylaxis.

2. MATERIALS AND METHODS

2.1. Animals:

In the present investigation, male albino mice weighing 25-30 g, male Sprague Dawley albino rats weighing 120-180 g and guinea pigs weighing 250-350g were used. Animals were obtained from the animal house of the National Research Center, Giza, Egypt. They were fed a standard pellet chow (El-Nasr Chemical Company, Cairo, Egypt) and had free access to water. All animals were maintained on a 12-h light, 12-h dark cycle and housed for 1 week before experimentation. This study was conducted in accordance with the ethical procedures and policies, approved by The Animal Care and Use Committee Faculty of Pharmacy and Biotechnology, German University in Cairo.

2.2. Chemicals:

TQ (2-isopropyl-5-methyl-1, 4-benzoquinone), available in a yellow crystalline form, was purchased from Sigma-Aldrich, Germany. TQ was dissolved in saline using water bath kept at 60°C. It was administered intraperitoneally in different doses; 3 mg /kg in guinea pigs in tracheal muscle preparation *(Gilani et al., 2001)*, 8 mg/kg in LPS-induced lung injury method *(El Gazzar et al., 2006)* in mice, 8mg/kg in rat peritoneal mast cells method *(Chakravarty, 1993)* and 10 mg/kg, 50 mg/kg, and 100 mg/kg *(Choi et al., 2006)* in systemic anaphylactic shock technique in mice. LPS (Escherichia coli lipopolysaccharide serotype 0111:B4; Sigma, St. Louis, MO) is dissolved in 0.3 ml saline. Bordetella pertussis vaccine was purchased from the holding company for biological products and vaccines (VACSERA). All chemicals and reagents were purchased from Sigma (St. Louis, MO, USA), unless otherwise specified.

2.3. Methods:

2.3.1. Tracheal Muscle Preparation:

This model was performed according to the method described by Costantin *et al.,(1965)*; and Burka and Saad *(1984)*. Guinea pigs were allocated randomly to three groups, each of 8 animals. Group 1: Control group, in which animals received 0.5 ml saline, i.p. for five consecutive days. Group 2: Sensitized group, in which animals were sensitized using 200 mg of OVA given as 100 mg i.p. and 100 mg S.C. into two sides on the back of the animal at days 0 and 14. Group 3: TQ treated group, in which the animals received a dose of 3mg/kg, i.p., in 0.5 ml saline, daily for five consecutive days before OVA sensitization. Groups 2 and 3 were given OVA. After 21 days, the animals were sacrificed by stunning and exsanguinations, the throat was opened, the trachea was removed, cut spirally, and suspended in Krebs-Henseleit solution (118 mM NaCl, 4.7 mM KCl, 1.2 mMMgSO4, 2.2 mM CaCl2, 24.9mM NaHCO3, 1.2 mM KHPO4, and 11.1 mM glucose; at pH 7.4) maintained at 370C and bubbled with carbogen (95% O2 & 5% CO2). The responses were displayed using Hugo Sachs Electronik-ACAD (HSE-ACAD), version 1.1.1.180 (Germany). The inhibitory effect of TQ on the tracheal muscle was investigated using a cumulative concentration-response curve of acetylcholine (Ach) and histamine - induced contraction of tracheal spirals at doses ranging from 10-9 M to 10-4 M.

2.3.2. LPS-Induced Acute lung injury (ALI):

LPS, a bacterial cell wall component, is a stimulus for the initiation of local acute inflammation. It is used for induction of a model for ALI. The method was performed according to Wang *et al., (2008)*. Mice were allocated randomly to three groups, each of 8 animals. Group 1: Control group, in which the animals received 0.3 ml saline i.p. Group 2: LPS-treated group, in which animals received 1 mg/ kg LPS dissolved in 0.3 ml saline i.p. Group 3: TQ treated group, in which the animals received TQ (8mg/kg) i.p. in 0.3 ml saline, 30 minutes before LPS injection. One hour later, the animals were anesthetized and bronchoalveolar lavage (BAL) was performed to collect BAL fluid (BALF) for measurement of total and differential inflammatory cell counts. BAL was performed through a tracheal cannula using 1.0 ml aliquots of ice-cold Ca2+/ Mg2+-free phosphate-buffered medium

(145 mM NaCl, 5 mM KCl, 1.9 mM NaH2PO4, 9.35 mM Na2HPO4, and 5.5 mM dextrose; at pH 7.4) for a total of 3 ml for each mouse. The recovered BALF was centrifuged at 300 g for 10 min at 4°C. The total number of cells was counted using a standard hemocytometer. Cell differentiation was examined by counting at least 200 cells on a smear prepared by using cytospin and Wright-Giemsa staining. Then, mice were sacrificed, chest opened, and lungs taken out and dried. Lung tissues were homogenized as 10%w/v in 1% KCl solution and stored at -80oC for measurements of oxidative stress parameters and inflammatory cytokines. Measurements of TNF-α, IL-1 β were done in BALF and lung homogenates while reduced glutathione (GSH) and malondialdehyde (MDA) contents were measured in lung homogenates.

2.3.2.1. Determination of TNF-α and IL-1β in BALF and lung homogenates:

BALF was centrifuged at 1000 xg for 20 minutes. Then the supernatant was kept at -80 °C for subsequent determination of TNF-α and IL-1β.The activity of TNF-α and IL-1β was later determined by enzyme-linked immunosorbant assay (ELISA) in accordance with to the manufacturer's recommended protocols, using Quantikine rats TNF-α/TNFSF1A and mouse IL-1β/IL-1β1F2 immunoassay commercial kits provided by R & D systems, Inc., Germany.

2.3.2.2. Estimation of reduced glutathione (GSH) in lung homogenates:

Glutathione content was estimated according to the method described by Tietze, *(1969)*.

Estimation of GSH contents was performed spectrophotometrically at 412 nm, using Ellman's reagent and expressed as mg/g wet tissue.

2.3.2.3. Estimation of malondialdehyde (MDA) content in lung homogenates:

The determination of MDA content was performed according to Mihara and Uchiyama, *(1978)*. The lipid peroxidation products were estimated in lung homogenates in ice cold saline by the determination of the level of thiobarbituric acid reactive substances (TBARS) that were measured as MDA. TBARS concentration was expressed as nmol MDA/ g wet weight.

2.3.3. Rat peritoneal mast cells preparation (RPMCs):

This model was carried out following the method described by Atkinson *et al.*, *(1979)*.

Male Sprague Dawley albino rats were actively sensitized with egg albumin according to the method of Chakravarty, *(1980)*. Rats were randomly classified into 3 groups, each of 6 animals. Group 1: Control group, in which animals received 0.5 ml saline i.p. Group 2: Sensitized group, in which animals were sensitized using a mixture of 0.25 ml 2% (w/v) egg albumin in 0.9% NaCl solution and 0.5 ml Bordetella pertussis vaccine (2 x 1010 bacilli) s.c. on the first day, a mixture of 0.25 ml 2% egg albumin and 0.5 ml Freund's incomplete adjuvant on the second day and 0.25 ml of the egg albumin solution only on the third day. Group 3:

TQ treated group, in which the sensitized animals received a dose of TQ 8mg/kg (i.p) daily for 21 days starting from the first day of sensitization. Aliquots of cells (1ml) from each of the 3 groups were allowed to equilibrate at 37°C for 5 minutes. A 100 μl of the solution of the releasing agent (compound 48/80) at a concentration of 0.2 μg / ml was added. Histamine release was allowed to proceed for a further 10 min and the reaction was terminated by the addition of 2 ml ice-cold Tyrode solution. Cells and supernatants were separated by centrifugation at 3000 rpm for 2 min at 4°C .The cell pellets were re-suspended in 3 ml Tyrode solution and allowed to stand in a boiling water bath for 10 min to release residual histamine. Individual supernatants were treated similarly. Histamine was then determined spectrofluorimetrically *(Atkinson et al., 1979)* using Vector multiple plate counter, ELISA reader. Histamine release was expressed as percentage of the total cellular content of the amine.

2.3.4. Systemic Anaphylactic Shock Induced by Compound 48/80 in mice:

This model was carried out according to the method described by Choi *et al.*, *(2006)*. Mice were randomly classified into 3 groups each of 10 animals; Group 1: Control group, in which animals received 0.5 ml saline i.p. Group 2: TQ (10 mg/kg, i.p). Group 3: TQ (50 mg/kg, i.p).Group 4: TQ (100 mg/kg, i.p). In groups 2, 3 &4, TQ was injected one hour before the injection of compound 48/80 (8 mg/kg, i.p.). Mortality rate was monitored for 1 h after the induction of anaphylactic shock.

2.3.5. Statistical analysis:

Data were expressed as the mean ± standard error (S.E.M). Statistical analysis was performed using prism software, version 5 (Graph pad Software, Inc., San Diego, USA). One-way analysis of variance (ANOVA) followed by Tukey-Kramer was used for comparing means of different groups. *P* values < 0.05 were considered statistically significant.

3. RESULTS

3.1. Effect of TQ on the tracheal muscle preparation of sensitized guinea pigs contracted with acetylcholine and histamine.

Sensitization of guinea pigs with OVA produced a significant increase in the sensitivity of the tracheal spirals contracted with Ach and histamine by about 3 folds and 2.5 folds, respectively, compared to the normal control group. Five days prior to sensitization, animals were pretreated with TQ (3mg/kg, i.p) daily. This produced a significant reduction in the sensitivity of the tracheal smooth muscle preparations to Ach and histamine by 71% and 74%, respectively as compared to the sensitized animals (Table 1; Figures 1 and 2)

3.2.1. Effect of LPS (1 mg/kg) and TQ (8 mg/kg) on the total and differential inflammatory cell counts in the BALF of mice.

LPS (1 mg/kg) produced a significant increase in the total count and the differential counts of inflammatory cells in BALF. LPS caused a significant increase in the number

of total as well as the eosinophils, neutrophils, macrophages and lymphocytes, as compared to the normal control values. Pretreatment with TQ (8 mg/kg) suppressed the number of total as well as the differential cell counts to values approaching the normal values (Table 2).

3.2.2. Effect of LPS (1 mg/kg) and TQ (8 mg/kg) on TNF-α and IL-1β levels in BALF and lung tissue homogenates of mice.

Treatment of mice with LPS (1 mg/kg, i.p.) significantly increased TNF-α and IL-1β in both BALF and lung homogenates by about one to two fold, as compared to the normal control value. Pretreatment with TQ (8 mg/kg, i.p.) ameliorated both TNF-α, IL-1β in BALF and lung homogenates to values that are significantly lower than the respective LPS-induced increments and approaching the normal control values (Figures 3,4,5&6).

3.2.3. Effect of LPS (1mg/kg) and TQ (8 mg/kg) on MDA, GSH oxidative stress indices in the lung homogenate of mice.

Treatment with LPS (1 mg/kg, i.p.) significantly increased MDA level in tissue homogenate by about one-fold compared to the normal control values. GSH content was almost decreased by 54%, as compared to the normal control value. Pretreatment with TQ (8 mg/kg, i.p.) suppressed the LPS-induced increase in MDA by 51% compared to the LPS treated mice. On the other hand, TQ ameliorated the LPS-induced depletion of GSH content by 86%, as compared to the LPS treated mice (Figures 7 &8).

3.3. Effect of TQ (8mg/kg, i.p.) on the percentage of histamine release from RPMCs of sensitized rats.

Sensitization of rats increased the percentage of histamine release from RPMCs by 94%, compared to the normal control group. TQ (8mg/kg, i.p.) produced a significant inhibition of the percentage of histamine release by 41%, as compared to the sensitized rats (Table 3).

3.4. Effect of TQ (10, 50, 100 mg/kg, i.p.) on the systemic anaphylactic shock induced by compound 48/80(8 mg/kg, i.p.) in mice.

Compound 48/80 (8 mg/kg, i.p.) produced a fatal shock in all mice. Pretreatment of mice with TQ (10, 50,100 mg/kg) for 1 h resulted in a dose-dependent reduction in the percentage of mortality with compound 48/80, compared to control mice receiving compound 48/80 only.

Table 1: Effect of TQ (3 mg/kg) on the maximal responses of acetylcholine and histamine on tracheal muscle preparation of normal and sensitized guinea pigs.

Treatment	Groups		
	Normal Control	Sensitized (OVA)	OVA +TQ (3 mg/kg)
Acetylcholine (10^{-9}-10^{-4}M)	20.12 ± 0.39	81.20 ± 0.97*	23.34 ± 0.60@ (71%)
Histamine (10^{-9}-10^{-4}M)	25.10 ± 0.24	85.43 ± 1.03*	22.21 ± 0.24@ (74%)

TQ (3 mg/kg, i.p.) was administered to guinea pigs for five consecutive days prior to OVA (200 mg given as 100 mg i.p. and 100mg S.C., at days 0 and 14). Sensitization lasted for 21 days.
Percentage inhibitions from the respective sensitized value are given in parentheses.
Results are expressed as the mean ± S.E, n = 8. Statistical analysis was carried out using one-way ANOVA followed by Tukey- Kramer multiple comparisons test.
*Significant difference from control group at $P < 0.05$.
@Significant difference from OVA sensitized group at $P < 0.05$.

Table 2: Effect of LPS and TQ on the total and differential inflammatory (eosinophils, neutrophils, macrophages and lymphocytes) cell counts in bronchoalveolar lavage fluid (BALF).

Parameters	Groups		
	Normal control	LPS (1mg/kg)	LPS (1 mg/kg) + TQ (8 mg/kg)
Total cell count ($\times 10^6$cells)	0.22 ± 0.011	0.56 ± 0.042*	0.26 ± 0.05@
Eosinophils ($\times 10^6$cells)	0.042 ± 0.006	0.201 ± 0.012*	0.089 ± 0.003@
Neutrophils ($\times 10^6$cells)	o.847 ± 0.03	2.218 ± 0.13*	1.002 ± 0.03@
Macrophages ($\times 10^6$cells)	0.398 ± 0.02	0.861 ± 0.043*	0.412 ± 0.035@
Lymphocytes ($\times 10^6$cells)	0.016 ± 0.004	0.109 ± 0.02*	0.029 ± 0.006@

Saline (10 ml/kg, i.p.), TQ (8 mg/kg, i.p.) were administered to mice, 30 minutes prior to LPS (1 mg/kg, i.p.).
Results are expressed as the mean ± S.E, n=8. Statistical analysis was carried out using one-way ANOVA followed by Tukey- Kramer multiple comparisons test.
*Significant difference from normal control group at $P < 0.05$.
@Significant difference from LPS-treated group at $P < 0.05$.

Table 3: Effect of TQ (8 mg/kg) on compound 48/80-induced histamine release from RPMCs.

	Groups		
	Normal control	**Sensitized**	**Sensitized + TQ (8 mg/kg)**
%Histamine release	42.78±0.19	83.17±0.13*	48.67±0.69 @

RPMCs from normal (10 ml/kg saline, i.p.), sensitized and sensitized rats, pretreated with TQ (8 mg/kg, i.p.), were incubated for 10 minutes at 37ºC with compound 48/80 (8mg/kg). % of histamine release was determined spectrofluorometrically.

Results were expressed as the mean ± S.E, n=6. Statistical analysis was carried out using one-way ANOVA followed by Tukey- Kramer multiple comparisons test.

*Significant difference from normal control group at *P* < 0.05.

@Significant difference from sensitized group at *P* < 0.05.

Table 4: Effect of TQ (10-50-100 mg/kg) on compound 48/80-induced systemic anaphylaxis in mice.

Treatment	Dose (mg/kg) i.p.	Mortality % at specified time in minutes			
		10	15	30	60
48/80	8	20	30	100	100
TQ	10	10	20	100	100
TQ	50	0	10	60	100
TQ	100	0	0	10	40

Groups of mice (n=10/group) were injected with saline (10 ml/kg, i.p.) or TQ (10-50-100 mg/kg, i.p.) 1 h before the injection of compound 48/80 (8 mg/kg, i.p.). Mortality (%) within 1 h following compound 48/80 injection was presented as the number of dead mice ×100/total number of experimental mice.
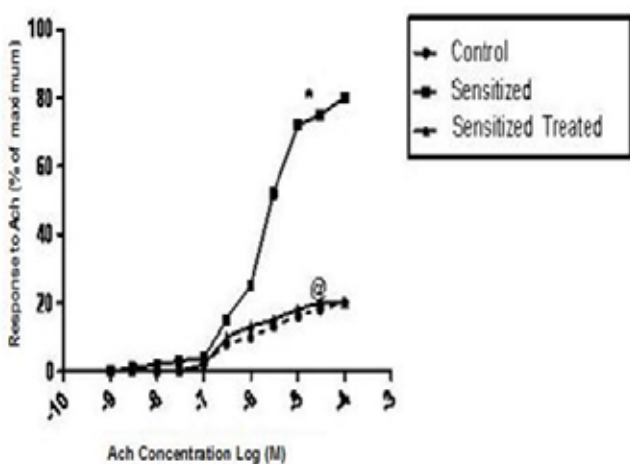

Figure 1: Effect of TQ (3 mg/kg) on the cumulative concentration-response curve of Ach- induced contraction on guinea pig tracheal spiral pre-sensitized with OVA.

Results were expressed as percentage of the maximal response of Ach.

TQ (3 mg/kg, i.p.) was administered to guinea pigs for five consecutive days prior to OVA (200 mg given as 100 mg i.p. and 100 mg S.C., at days 0 and 14). Sensitization lasted for 21 days.

Results are expressed as the mean ± S.E, n=8. Statistical analysis was carried out using one-way ANOVA followed by Tukey- Kramer multiple comparisons test.

*Significant difference from normal control group at *P* < 0.05.

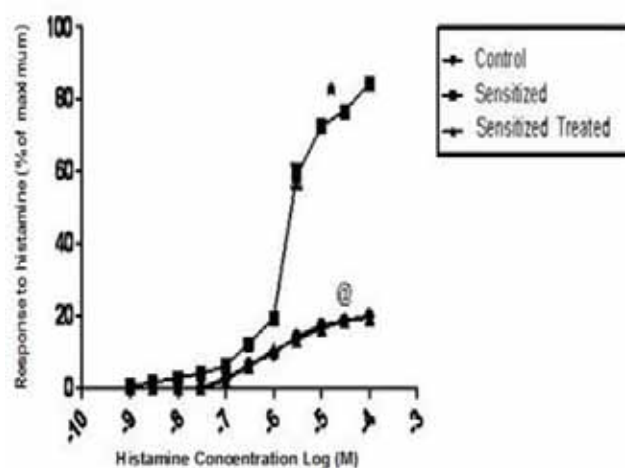@Significant difference from ovalbumin sensitized group at *P* < 0.05.

Figure 2: Effect of TQ (3 mg/kg) on the cumulative concentration-response curve of histamine-induced contraction of guinea pig tracheal spiral pre-sensitized with OVA.

Results are expressed as percentage of maximal response of histamine.

TQ (3 mg/kg, i.p.) administered to guinea pigs for five consecutive days prior to OVA (200 mg given as 100 mg i.p. and 100 mg S.C., at days 0 and 14). Sensitization lasted for 21 days.

Results are expressed as the mean ± S.E, n=8. Statistical analysis was carried out using one-way ANOVA followed by Tukey- Kramer multiple comparisons test.

*Significant difference from normal control group at *P* < 0.05.

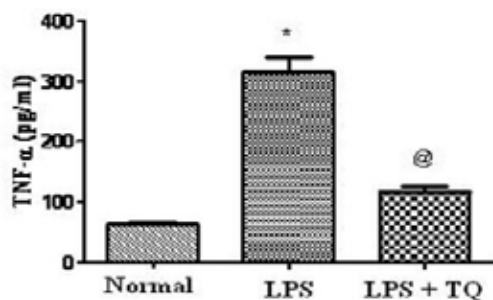@Significant difference from ovalbumin sensitized group at *P* < 0.05.

Figure 3: Effect of LPS (1 mg/kg) and TQ (8 mg/kg) on TNF- α level in BALF of mice.
Saline (10 ml/Kg, i.p.) and TQ (10 mg/kg, i.p.) were administered to mice, 30 minutes prior to LPS (1 mg/kg, i.p.).
Results are expressed as the mean ± S.E, n = 8. Statistical analysis was carried out using one-way ANOVA followed by Tukey- Kramer multiple comparisons test.
*Significant difference from normal control group at $P < 0.05$.
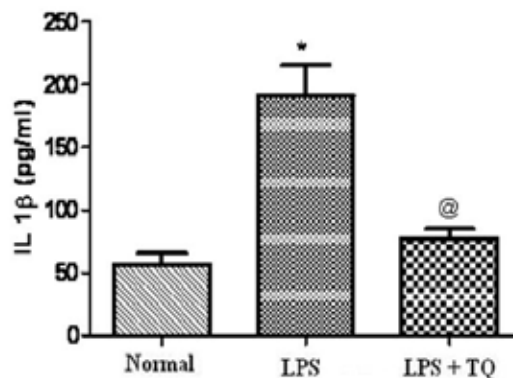@Significant difference from LPS-treated group at $P < 0.05$.

Figure 4: Effect of LPS (1 mg/kg) and TQ (8 mg/kg) on IL-1β level in BALF of mice.
Saline (10 ml/Kg, i.p.), and TQ (8 mg/kg, i.p.) were administered to mice, 30 minutes prior to LPS (1 mg/kg, i.p.).
Results are expressed as the mean ± S.E, n = 8. Statistical analysis was carried out using one-way ANOVA followed by Tukey- Kramer multiple comparisons test.
*Significant difference from normal control group at $P < 0.05$.
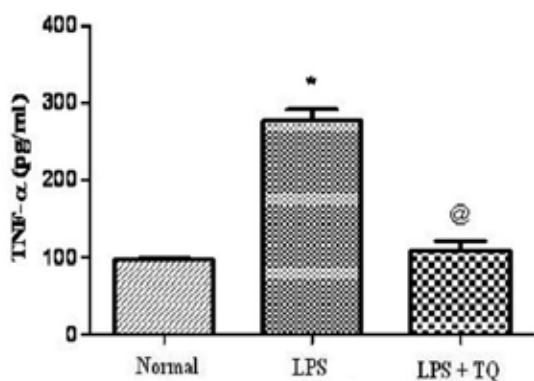@Significant difference from LPS-treated group at $P < 0.05$.

Figure 5: Effect of LPS (1 mg/kg) and TQ (8 mg/kg) on the TNF-α level in the lung homogenates of mice.
Saline (10 ml/Kg, i.p.) and TQ (8 mg/kg, i.p.) was administered to mice, 30 minutes prior to LPS (1 mg/kg).
Results are expressed as the mean ± S.E, n = 8. Statistical analysis was carried out using one-way ANOVA followed by Tukey- Kramer multiple comparisons test.
*Significant difference from normal control group at $P < 0.05$.
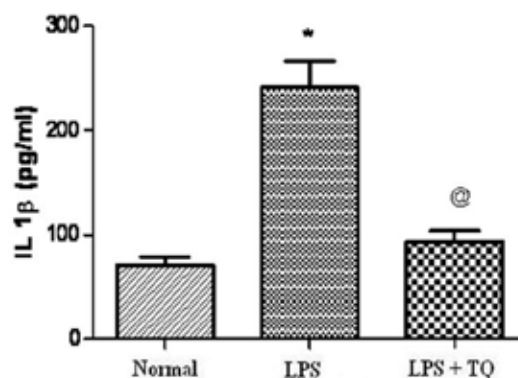@Significant difference from LPS-treated group at $P < 0.05$.

Figure 6: Effect of LPS (1 mg/kg) and TQ (8 mg/kg) on the IL-1β level in the lung homogenates of mice.
Saline (10 ml/Kg, i.p.) and TQ (8 mg/kg, i.p.) was administered to mice, 30 minutes prior to LPS (1 mg/kg).
Results are expressed as the mean ± S.E, n = 8. Statistical analysis was carried out using one-way ANOVA followed by Tukey- Kramer multiple comparisons test.
*Significant difference from normal control group at $P < 0.05$
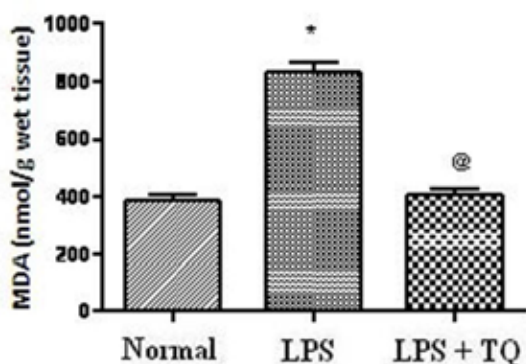@Significant difference from LPS-treated group at $P < 0.05$.

Figure 7: Effect of LPS (1 mg/kg) and TQ (8 mg/kg) on MDA content in lung tissue of mice.
Saline (10 ml/kg, i.p.) and TQ (8 mg/kg, i.p.) were administered to mice, 30 minutes prior to LPS (1 mg/kg).
Results are expressed as the mean ± S.E, n = 8. Statistical analysis was carried out using one-way ANOVA followed by Tukey- Kramer multiple comparisons test.
*Significant difference from normal control group at $P < 0.05$.
@Significant difference from LPS-treated group at $P < 0.05$.

Figure 8: Effect of LPS (1 mg/kg) and TQ (8 mg/kg) on reduced glutathione (GSH) in lung tissue of mice.
Saline (10 ml/kg, i.p.) and TQ (8 mg/kg, i.p.) were administered to mice, 30 minutes prior to LPS (1 mg/kg).
Results are expressed as the mean ± S.E, n = 8. Statistical analysis was carried out using one-way ANOVA followed by Tukey- Kramer multiple comparisons test.
*Significant difference from normal control group at $P < 0.05$.
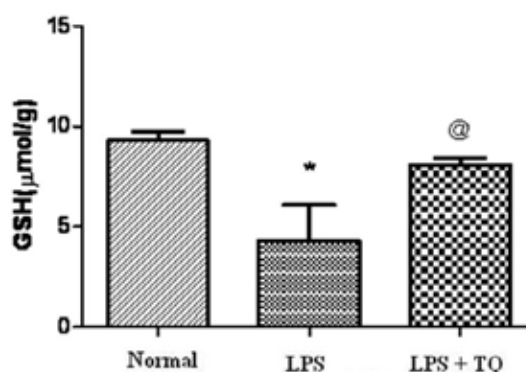@Significant difference from LPS- treated group at $P < 0.05$.

4. DISCUSSION

In the present study, pretreatment of the sensitized guinea pigs with TQ showed a significant decrease in the response of the tracheal spirals to histamine and acetylcholine compared to that produced with sensitized animals. The traditional use of Nigella sativa seeds and its active ingredients have a substantial impact on the inflammatory diseases mediated by histamine *(Kalus et al., 2003)*. These results were supported by previous studies that investigated TQ's effect on the guinea pig isolated tracheal zig-zag preparation pre-contracted by carbachol. TQ caused a concentration-dependent decrease in the tension of the tracheal smooth muscle *(Al-Majed et al., 2001)*. The TQ - induced relaxation is probably mediated, at least in part, by inhibition of lipoxygenase (LO) products of arachidonic acid metabolism and possibly by non-selective blocking of the histamine and serotonin receptors. This relaxant effect of TQ, further support the traditional use of black seeds to treat bronchial asthma *(Al-Majed et al., 2001)*. However, in an in vivo study, increasing respiratory rate and intra tracheal pressure of guinea pigs due to i.v. administration of volatile oil from Nigella sativa has been demonstrated. These respiratory effects were mediated via release of histamine with direct involvement of histaminergic mechanisms and indirect activation of muscarinic cholinergic mechanisms *(El-Tahir et al., 1993)*. In previous studies, the relaxant, anticholinergic (functional antagonism) and antihistaminic, effects of Nigella sativa have been demonstrated on guinea pig tracheal chains *(Boskabady and Shahabi, 1997; Boskabadi and Shiravi, 2000)*. This relaxant effect is not attributed to the calcium channel blocking effect of Nigella sativa extracts. However, a potassium channel opening effect was suggested for its extracts *(Boskabady et al., 2004)*. Results of a recent study showed a preventive effect of thymoquinone on tracheal responsiveness and inflammatory cells of lung lavage of sensitized guinea pigs which was comparable or even greater than that of the inhaled steroid *(Keyhanmanesh et al., 2010)*. The reduction in the response of the tracheal spirals to acetylcholine and histamine could be also due to a direct inhibitory effect of TQ on the release of mediators involved in chronic airway inflammation including histamine, serotonin and bradykinin. Those mediators are known to be responsible for the increased vascular permeability and blood flow observed during the early stages of inflammation *(kumar et al., 2007)*.

In the present investigation, injection of LPS increased inflammatory total and differential cells count namely, eosinophils, neutrophils, macrophages and lymphocytes both in BALF and lung homogenates. It increased the oxidative stress by increasing lipid peroxidation and decreasing GSH levels. Similarly, it stimulated cytokines; TNF-α and IL-1β production. Pretreatment with TQ (8 mg/kg) prevented most of the pathological detrimental changes that occurred in response to the endotoxin LPS. TQ reduced inflammatory cell infiltration into respiratory airways, oxidative stress in terms of lipid peroxidation and increased GSH levels and decreased TNF-α and IL-1β production.

The main pathological feature of ALI/ARDS is pulmonary edema brought about by aggregation of pulmonary neutrophils and increased permeability of alveolar-capillary membrane *(Balibrea and Arias-Dlaz, 2003)*. In a previous study, it was found that TQ improved oxygenation while both TQ and steroids protect lung tissue from hazardous effects of human gastric juice histopathologically in a rat model of ALI/ARDS *(Isik et al., 2005)*.

Oxidative stress has been shown to play a major role in mediating lung injury both in animal models and ALI/ARDS patients. Oxygen radicals were found to be responsible for LPS-induced lung injury *(Feng et al., 2004)*. Pulmonary function was improved in ARDS patients in response to N-acetylcysteine antioxidant therapy *(Bernard, 1990)*. Asti et al., *(2000)* have demonstrated that LPS treatment in mice resulted in acute hemorrhagic lung injury with increased neutrophil infiltration and increased lung MPO activity. Activation of the neutrophil NADPH oxidase leads to liberation of reactive oxygen species (ROS) *(Chanock et al., 1994)*. ROS have been implicated in tissue injury associated with inflammation, organ ischemia and reperfusion, ARDS, rheumatoid arthritis and asthma *(Smith, 1994)*. O2•⁻ contributes to the inflammatory response through several mechanisms including lipid peroxidation, increase of vascular permeability, cellular recruitment and tissue damage *(Boueiz and Hassoun, 2009)*. It was found to contribute to the inflammatory process via different pathways including lipid peroxidation, enzymatic inactivation *(Crow and Beckman, 1995)*, glutathione depletion *(Phelps et al., 1995)* and DNA damage *(Inoue and Kawanishi, 1995)*.

Recent study showed that LPS-administered rats with TNF-α blocking peptide significantly suppressed the levels of pulmonary endothelin (ET-1) and that differential alteration in ET expression may be mediated by TNF-α and may, in part, account for the pathogenesis of acute lung injury in endotoxemia *(Jesmin et al., 2011)*. TNF-α and IL-1β mediate the neutrophil migration observed in several experimental models and also in human inflammatory disease *(Gong et al., 2009)*. TNF-α through induction of neutrophils adhesion and their subsequent activation, mediates neutrophil-dependent increase in vascular permeability *(Lentsch and Ward, 2000)*.

El Gazzar *et al*. *(2006)* proved that TQ attenuated pulmonary inflammation in a mouse model of allergic asthma by decreasing Th-2 cytokines and inflammatory cell infiltration in the lung. Attenuation of cellular recruitment observed with TQ can lead to subsequent reduction of oxidative and/or nitrosative stresses and their accompanied deleterious effects including increased vascular permeability, lipid peroxidation, GSH depletion and tissue damage. Moreover, TQ improved renal GSH depletion and lipid peroxides accumulation in ifosfamide-induced renal damage *(Badary, 1999)*.

TQ was shown to inhibit cyclooxygenase (COX) and 5- lipooxygenase (5-LOX) pathways in rat peritoneal leukocytes stimulated with calcium ionophore A23187 *(Houghton et al., 1995)*. Another study showed that TQ attenuates the inflammatory response in activated mast cells by blocking transcription and production of TNF-α. TQ exerted its effects by targeting the nuclear transactivation of

pro-inflammatory transcription factor nuclear factor-kappa B (NF-κB) *(El Gazzar et al., 2007)*. Another study proved that licorice flavonoids effectively attenuate LPS-induced acute pulmonary inflammation in mice through inhibition of inflammatory cells infiltration and inflammatory mediator release. This subsequently, reduces neutrophil recruitment into lung and neutrophil-mediated oxidative injury. This may be achieved through reduction of LPS-induced lung TNF-α and IL-1β mRNA expression, increasing SOD activity *(Xie et al., 2009)*.

It was also reported that TQ tends to decrease the elevated levels of LPS-induced TNF-α in macrophages from diabetic rats *(El-Mahmoudy et al., 2005)*.

The effect of TQ on TNF-α was previously investigated. El Gazzar *et al.* (2007) showed that TQ attenuated the proinflammatory response in LPS-stimulated mast cells by blocking transcription and production of TNF-α. Tekeoglu *et al.* (2006) also reported that TQ exerted an inhibitory effect on TNF-α production in rheumatoid arthritis, a well established chronic inflammation model in rats.

Adrenomedullin was shown to protect rats from LPS-induced ALI. Similar to thymoquinone, adrenomedullin decreased the total cells and neutrophils count and reduced the TNF-α levels *(Itoh et al., 2007)*. Additionally, statins were shown to protect against LPS-induced lung injury through reduction of inflammatory cell infiltration and decreased cytokines production namely TNF-α and IL-6 *(Jacobson et al., 2005; Yao et al., 2006)*. Moreover, it was proved that the free radical scavenger, edaravone, is able to attenuate LPS-induced acute lung injury in mice via suppression of pro-inflammatory cytokine production by lung macrophages *(Tajima et al., 2008)*. The causal link between oxidative stress and cytokine production was shown in vitro in alveolar epithelium. Inhibition of GSH biosynthesis by buthionine sulfoximine increased intracellular oxidative stress and enhanced the production of IL-1β, IL-6, and TNF-α *(Haddad et al., 2001)*.

The anti-allergic effect of TQ was evaluated by its reduction of the release of histamine from the rat peritoneal mast cells and by decreasing the mortality of mice in the systemic anaphylaxis reaction induced by compound 48/80.

Pretreatment with TQ inhibited the elicited increase in histamine release compared to the sensitized group. These results clearly indicated that TQ inhibited mast cell-mediated immediate-type allergic reactions. It was reported that rats pretreated with Nigella sativa oil before induction of ulcer caused a significant decrease in gastric mucosal histamine content *(El-Dakhakhny et al., 2000)*.

Previous reports showed that nigellone was very effective in inhibiting histamine release induced by the secretagogues: antigen in sensitized cells, compound 48/80, and the calcium ionophore A23187 through decreasing intracellular calcium by inhibiting its uptake and stimulating the efflux, and by an inhibition on protein kinase C. There is also indication for a mild inhibition of oxidative energy metabolism

contributing to some inhibition of the release *(Chakravarty, 1993)*. Moreover, Gilani *et al.*, (2001) found that the crude extract of Nigella sativa seeds exhibits spasmolytic and bronchodilator activities mediated possibly through calcium channel blockade. Recent studies done on curcumin and α-lipoic acid showed that the inhibition of mediator release from RPMCs may be due to inhibition of calcium uptake and augmentation of intracellular cAMP levels *(Choi et al., 2010 a, b)*. These findings together with the known anti-oxidant properties of TQ suggest that it could be used in the treatment of immediate-type allergic diseases.

It is well-recognized that compound 48/80 can induce a mast cell-dependent, non-specific anaphylactoid reaction. Compound 48/80 is known to activate mast cell secretory processes by increasing the rate of guanosine triphosphate-gamma S (GTP)γS binding to G-proteins *(Palomäki and Laitinen, 2006)*. This, in turn, triggers activation of PKC and Ca2+ signaling and results in the release of histamine from these cells. TQ potently suppressed histamine release probably through the inhibition of the degranulation process following a rise in intracellular Ca2+ levels, in accordance with previous reports *(Suzuki et al., 2005; Nugroho et al., 2009)*.

From the previous findings, we can conclude that TQ possesses marked anti-allergic and anti- asthmatic activity and may have beneficial effects in the prevention or treatment of many allergic diseases.

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