Current Science International Volume : 04 | Issue : 04| Oct.-Dec.|2015
ISSN 2077-4435 Pages: 538-547

Protective and Anti-oxidant Effects of Thymoquinone against Asthma Sensitized by Aspergillus fumigatus in Albino Rats

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ABSTRACT

Thymoquinone (TQ), the main active constituent of the volatile oil extracted from *Nigella sativa* seeds, is used for the treatment of inflammatory diseases and exhibits a variety of antioxidant and anti-inflammatory modulatory effects. The present study aims to investigate the ameliorative effect of TQ on bronchial asthma in male albino rats that sensitized by *Aspergillus fumigatus* spores (AFs) by evaluating the anti-oxidant activity of TQ. It was found that TQ (0.1 µ g/kg b.wt.) increased the levels of Glutathione Peroxidase (GPx), Glutathione-S-Transferase (GST), Glutathione Reduced (GSH), Glutathione Reductase (GR), while, it decreased the levels of superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA). Also, TQ alone decreased the serum total protein concentration. Considering the anti-asthmatic and antioxidant effects of TQ reported in this study, one can conclude that TQ could be of modulator potentials on bronchial asthma.

Key words: Thymoquinone, Aspergillus fumigatus, bronchial asthma, oxidative stress, rats.

Introduction

Oxidative stress describes the damage that occurs when oxidants overwhelm the antioxidant system, and may play an important role in the pathophysiology of asthma (Chanez *et al*., 1990). Asthma is a chronic, relapsing inflammatory disorder characterized by hyper-reactive airways (Cotran *et al*., 1999 & Porth, 1990) and it is associated with tissue remodeling of the airway structure (Jenna *et al*., 2010). Inflammatory cells that infiltrate the airways produce several modulators of the inflammatory response, including a range of toxic reactive oxygen species (ROS) (Rice and Burdon, 1993) that associated with many of the pathophysiological changes linked with asthma (Barnes, 1990).

Most environmental factors including allergic responses to allergens such as *Aspergillus fumigatus* act as oxidative stress upon cells (Bowler and Crapo, 2002). To maintain a steady state, airway epithelial cells possess mechanisms that eliminate oxygen radicals (Nakamura *et al*., 1997). Understanding how respiratory diseases are exacerbated requires consideration of the effect of inflammation as an oxidative stress.

The body has its own antioxidant defense mechanisms to stabilize oxidative molecules and keep them in balance. Cells are equipped with endogenous antioxidants, either enzymatic or non-enzymatic which are crucial for preventing or at least slowing the incidence and progression of diseases (Jacob, 1995).

Protein oxidation and lipid peroxidation, all of these oxidative changes can be cumulated in airway and may participate to bronchial asthma persistence and lead to further release of mediators from epithelium resulting in further increase of oxidative damage which can again participate in asthma pathogenesis. The levels of this oxidative damage can be controlled *in vivo* by antioxidant enzymes which are considered to be the first line of cellular defense against oxidative damage (Nakbi *et al*., 2010). Among them, superoxide dismutase (SOD) which is universal enzymatic antioxidant. This enzyme is extremely efficient and catalyses the neutralization of superoxide anion to oxygen and hydrogen peroxide (Yildiz *et al*., 2008), catalase (CAT) which is a common antioxidant enzyme responsible for controlling hydrogen peroxide concentrations in cells. Catalase as an intracellular antioxidant enzyme catalyzes the decomposition of two molecules of hydrogen peroxide into one molecule of oxygen and two of water (Yildiz *et al*., 2008), glutathione peroxidases (GPXs) which are family of enzymes ubiquitously distributed which have peroxidase activity whose a main biological role is to protect the organism from oxidative damage. Glutathione peroxidases reduce hydrogen peroxide to water and reduced glutathione and lipid hydroperoxides to their corresponding alcohols, water and reduced glutathione (Tappel, 1984), glutathione Reductase (GR) which participates on maintenance of intracellular concentration of glutathione (Ruscoe *et al*., 2001), glutathione transferases (GSTs) that inactivate endogenous unsaturated aldehydes, quinone, epoxides, and hydroperoxides formed as secondary metabolites during oxidative damage. GSTs may reduce reactive oxygen species to less reactive metabolites and protect organism against consequences of lipid peroxidation (Ruscoe *et al*., 2001) and glutathione reduced GSH which is an important antioxidant which reduces organic hyperoxides and protects organs from lipid peroxidation (Ames *et al*., 1993).

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Antioxidants from plants are reported to provide substantial protection that slows down the process of oxidative damage caused by reactive oxygen species (ROS) (Jacob and Burri, 1996). Among the promising medicinal plants, *N. sativa*, which is belonging to the Ranuculacease family, is an amazing herb with rich historical and religious background. The seeds of *N. sativa* are the source of thymoquinone (TQ) which is the active ingredient of this plant (Salem, 2005). The seeds of *N. sativa* known as black seeds have long been used in folk medicine for a wide range of illnesses, including bronchitis, asthma, diarrhea, rheumatism and skin disorders. It is also used as liver tonic, digestive, anti-diarrheal, appetite stimulant, to increase milk production in nursing mothers to fight parasitic infections, and to support immune system (Al-Rowais, 2002) due to the antioxidant and anti-inflammatory effects of TQ (Gali-Muhtasib *et al*., 2008). The most important active compounds of seeds of *N. sativa* are thymoquinone (30%-48%), thymohydroquinone, dithymoquinone, p-cymene (7%-15%), carvacrol (6%-12%), 4-terpineol (2%-7%), t-anethol (1%-4%), sesquiterpene longifolene (1%-8%) α-pinene and thymol *etc*. Black seeds also contain some other compounds in trace amounts (Al-Jassir, 1992).

So that, the goal of this work is to investigate the ameliorative effect of thymoquinone against the oxidative stress that potentiated by some allergens such as the fungs *Aspergillus fumigates* which acts as an initiative agent for pulmonary asthma in albino rats.

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 standard 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 haemocytometer. The suspension was then diluted to the desired concentration, and the concentration of spores was again measured before administration (Nascimento *et al.,* 2010).

Experimental design

Eighteen adult male albino rats (*Rattus norvegicus*), weighing 100-120 g, were used in preliminary study to determine the period of *Aspergillus fumigatus* administration and this preliminary study indicated that symptoms of allergic asthma like enphyzema begin to appear at the 4th week post-injection. In the actual study, animals were grouped into eleven groups; the first group was normal (injected free) (N_0), five groups were control and the other five groups were treated, where each group consisted of eighteen animals. The first control group (C_1) was injected intranasal once every three days for 6 weeks with 0.05 µ g/ kg b.wt. of Phosphate Buffer Saline (PBS) which was the suspending medium of *Aspergillus fumigatus* spores. The second control group (C_2) was injected intraperitonealy once every three days for 6 weeks with 0.1 µ g/kg b.wt. of TWEEN 20 which was the solvent of thymoquinone. The third control group (C_3) was injected intranasal with 0.05 µ g/kg b.wt. of PBS and intraperitonealy with 0.05 µ g/kg b.wt. of TWEEN 20 at the same time once every three days for 6 weeks. The fourth control group (C_4) was injected intranasal once every three days for 4 weeks with 0.05 µ g/kg b.wt. of PBS, then , it was injected intraperitonealy once every three days for another 2 weeks with 0.1 µ g /kg b.wt. of TWEEN 20. The fifth control group (C_5) was injected intraperitonealy once every three days for 2 weeks with 0.1 µ g/kg b.wt. of TWEEN 20, then , it was injected intranasal once every three days for another 4

weeks with 0.05 µ g/kg b.wt. of PBS. The first treated group (T_1) was injected intranasal once every three days for 6 weeks with 0.05 µ g/kg b.wt. of *Aspergillus fumigatus* spores suspension. The second treated group (T_2) was injected intraperitonealy once every three days for 6 weeks with 0.1 µ g/kg b.wt. of thymoquinone. The third treated group (T_3) was injected intranasal with 0.05 µ g/kg b.wt. of *Aspergillus fumigatus* spores suspension and intraperitonealy with 0.05 µ g/kg b.wt. of thymoquinone at the same time once every three days for 6 weeks. The fourth treated group (T_4) was injected intranasal once every three days for 4 weeks with 0.05 µ g/kg b.wt. of *Aspergillus fumigatus* spores suspension, then it was injected intraperitonealy once every three days for another 2 weeks with 0.1 µ g/kg b.wt. of thymoquinone. The fifth treated group (T_5) was injected intraperitonealy once every three days for 2 weeks with 0.1 µ g/kg b.wt. of thymoquinone, then , it was injected intranasal once every three days for another 4 weeks with 0.05 µ g/kg b.wt. of *Aspergillus fumigatus* spores suspension (Table 1).

Table 1: Configuration for the plan of the experiment

Time/weeks / Groups	W1	W2	W3	W4	W5	W6
N_0	F-I	F-I	F-I	F-I	F-I	F-I
C_1(PBS)	$PBS^{(IN)}$	PBS	PBS	PBS	PBS	PBS
C_2(TW20%)	$TW20^{(IP)}$	TW20	TW20	TW20	TW20	TW20
C_3(PBS+TW20%)	$PBS^{(IN)}$+$TW20^{(IP)}$	PBS+TW20	PBS+TW20	PBS+TW20	PBS+TW20	PBS+TW20
C_4(PBS-TW20%)	$PBS^{(IN)}$	PBS	PBS	PBS	$TW20^{(IP)}$	TW20
C_5(TW20%-PBS)	$TW20^{(IP)}$	TW20	$PBS^{(IN)}$	PBS	PBS	PBS
T_1(AF)	$AF_s^{(IN)}$	AF_s	AF_s	AF_s	AF_s	AF_s
T_2(TQ)	$TQ^{(IP)}$	TQ	TQ	TQ	TQ	TQ
T_3(AF+TQ)	$AF_s^{(IN)}$+$TQ^{(IP)}$	AF_s+TQ	AF_s+TQ	AF_s+TQ	AF_s+TQ	AF_s+TQ
T_4(AF-TQ)	$AF_s^{(IN)}$	AF_s	AF_s	AF_s	$TQ^{(IP)}$	TQ
T_5(TQ-AF)	$TQ^{(IP)}$	TQ	$AF_s^{(IN)}$	AF_s	AF_s	AF_s

F-I= Free-Injection (IN)= Intra-nasal injection (IP)= Intra-peritoneal injection PBS= Phosphate buffer saline TW20= TWEEN20 AF_s= Aspergillus fumigatus spores TQ= Thymoquinone

Collection of blood samples

After each duration, rats of control and treated groups were sacrificed and two blood samples were immediately collected. The first sample was collected in heparinized tube (2.25µ heparine / 5 ml blood) for separation of blood plasma. The second sample was collected in non–heparinized tube for separation of blood serum. Both were centrifuged for 5 minutes at 10000 rpm and stored at (-80ºC) for biochemical analysis. Samples of plasma were used in determination of Glutathione-s-transferase (GST) activity and samples of serum were used in determination of Total Protein concentration, Superoxide dismutase (SOD) activity, Catalase (CAT) activity, Lipid peroxide (malondialdehyde), Glutathione reduced (GSH) concentration and Glutathione reductase (GR) activity,

Preparation of erythrocyte lysate

After obtaining of the plasma samples, the white buffy layer was removed and discarded, the erythrocytes were lysed in 4 times its volume of ice-cold HPLC grade water, centrifuged at 4,000 rpm for 15 minutes, the supernatant (erythrocyte lysate) was collected and stored at (-80 °C) for Glutathione peroxidase (GPx) activity.

Biochemical Measurements

Total protein concentration, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), malondialdehyde (MDA), glutathione reduced (GSH), glutathione reductase (GR) activities were determined spectrophotometrically according to the methods described by Bishop *et al*. (2000), Nishikimi *et al*. (1972)**,** Johansson & Borg (1988), Paglia& Valentine (1967)**,** Habig & Jakoby (1974), Ohkawa*et al*. (1979), Beutler*et al*. (1963),and Goldberg & Spooner (1983), respectively.

Statistical Analysis

Statistical analysis of the present data was performed throughout one way analysis of variance (ANOVA test). The results were expressed and drawn using the mean± standard error (M± SE) and differences were considered to be significant at ($P< 0.05$), highly significant at ($P<0.01$) and very highly significant at ($P<0.001$).

Results

Total protein concentration

The normal value of the serum total protein concentration was between (0.72±0.00) and (0.73±2.52).

Through the intranasal injection of *Aspergillus fumigatus* (AF) either before, without or combined with thymoquinone(TQ), the serum total protein concentrations were increased allover the experimental period

exceptat group (T_3) in the 4th week post-injection where the serum total protein concentration was slightly decreased. On the other hand, through the intraperitoneal injection of thymoquinone alone, the serum total protein concentration was very highly significant decreased all over the experimental period. Also, administration of thymoquinone with *Aspergillus fumigatus*at the same time decreased the serumtotal protein concentration significantly in the 4th week post-injection whereas administration of thymoquinone either after or before *Aspergillus fumigatus* increased the serum total protein concentration (Table 2).

Table 2: Effect of thymoquinone on serum total protein concentration in *Aspergillus fumigatus* sensitized albino rats (g/dl).

Time/Week / Groups	W1	W2	W3	W4	W5	W6
N_0	0.72±0.00	0.72±1,00	0.73±2.52	0.72±5.77	0.73±1.16	0.72±2.08
C_1(PBS)	1.02±1.04	1.05±5.77	1.08±2.08	0.99±1.00	1.03±3.37	0.99±1.16
C_2(TW20%)	1.20±1.16	1.10±1.33	1.00±1.00	0.97±2.08	0.95±1.53	0.99±2.52
C_3(PBS+TW20%)	1.04±2.60	1.01±5.77	1.02±2.52	1.03±1.16	1.02±1.67	1.02±1.00
C_4(PBS-TW20%)	0.74±8.82	0.71±2.33	0.68±1.53	0.70±2.52	0.69±3.51	0.69±1.00
C_5(TW20%-PBS)	0.80±1.00	0.97±3.33	1.15±1.16	1.27±3.33	1.04±8.82	1.02±4.33
T_1(AF)	1.06±1.16*	1.12±0.00***	1.09±1.00**	1.07±2.08***	1.31±1.20**	1.07±3.18***
T_2(TQ)	0.75±2.52***	0.73±1.53***	0.70±2.08***	0.71±1.00***	0.76±0.00***	0.76±1.16***
T_3(AF+TQ)	1.04±1.00	1.06±3.33***	1.08±1.16***	1.01±3.06*	1.02±2.19	1.07±3.18***
T_4(AF-TQ)	0.96±6.67***	0.95±2.08***	0.95±2.52***	1.11±1.45***	0.91±4.16***	1.03±1.86***
T_5(TQ-AF)	0.98±3.18***	0.98±3.71	0,96±2.52***	0.91±3.33***	1.00±3.33***	1.02±1.00

Superoxide Dismutase (SOD) Activity

The normal value of the SOD activity was between (396.58±1.53) and (425.30±1.97).

Through the whole experiment, SOD activity was decreased at all treated groups exceptat group (T_4) in the 2nd week post-injection where the SOD activity was increased (Table 3).

Table 3: Effect of thymoquinone on Superoxide Dismutase (SOD) activity in Aspergillus fumigatus sensitized albino rats (U/ml).

Time/week / Groups	W1	W2	W3	W4	W5	W6
N_0	396.75±5.72	400.44±5.84	425.30±1.97	421.93±1.54	412.87±3.90	396.58±1.53
C_1(PBS)	468.75±3.87	436.01±17.40	449.77±9.37	479.91±2.58	459.88±5.58	440.48±8.39
C_2(TW20%)	325.00±1.29	343.70±3.15	383.04±3,87	280.36±6.44	298.21±7.73	292.26±3.24
C_3(PBS+TW20%)	712.80±3.24	683.04±6.44	712.41±3.31	691.96±2.58	667.41±3.87	589.29±3.87
C_4(PBS-TW20%)	451.95±5.15	352.68±5.16	402.53±17.40	366.82±22.55	330.36±7.73	305.80±6.44
C_5(TW20%-PBS)	305.11±2.87	305.80±2.58	304.32±4.52	290.92±1.97	305.80±6.44	295.39±1.97
T_1(AF)	406.10±3.24***	345.98±3.87**	323.66±6.44***	361.61±3.87***	400.30±4.52**	399.59±4.48*
T_2(TQ)	277.53±1.97***	276.18±1.67***	266.37±1.97***	262.99±1.41	257.44±3.24**	290.18±2.58
T_3(AF+TQ)	659.22±17.40*	595.98±19.33*	589.88±16.79**	573.66±12.89**	529.02±6.44***	588.75±18.91
T_4(AF-TQ)	363.25±24.18*	364.58±23.84	355.66±9.67	335.29±8.78	306.55±7.10	299.11±3.87
T_5(TQ-AF)	280.45±0.72**	283.48±1.29**	234.38±9.02**	245.54±2.58***	266.37±3.24**	254.46±2.58***

Catalase (CAT) Activity

The normal value of the CAT activity was between (251.38±5.79) and (256.75±1.98).

Through the whole experiment, CAT activity was decreased at all treated groups. The decrease was very highly significant at groups (T_2) and (T_5) (Table 4).

Table 4: Effect of thymoquinone on Catalase (CAT) activity in *Aspergillus fumigatus* sensitized albino rats (U/L).

Time/week / Groups	W1	W2	W3	W4	W5	W6
N_0	253.69±5.68	251.38±5.79	256.75±1.98	252.74±1.63	254.64±3.87	253.77±1.53
C_1(PBS)	327.43±3.75	370.82±17.34	350.93±948	385.57±2.74	348.83±5.57	354.47±8.39
C_2(TW20%)	289.93±1.45	255.95±2.70	301.10±3.72	312.59±6.17	329.06±7.80	307.25±3.24
C_3(PBS+TW20%)	416.65±3.35	390.04±6.77	368.14±3.32	422.48±2.60	434.16±3.93	446.18±3.87
C_4(PBS-TW20%)	330.36±5.19	375.65±5.16	357.20±15.38	379.72±22.55	373.39±7.73	356.37±6.44
C_5(TW20%-PBS)	280.13±2.91	245.87±2.58	334.17±4.77	325.57±1.95	327.45±3.29	324.35±1.97
T_1(AF)	306.48±3.19*	300.59±3.69*	241.22±6.44**	215.22±4.18***	124.23±4.81***	263.53±4.79**
T_2(TQ)	160.02±2.08***	150.72±1.74***	143.70±1.78***	155.72±1.45***	169.56±3.58***	149.16±2.50***
T_3(AF+TQ)	320.20±17.14**	344.61±19.34	326.73±16.79	309.06±12.70**	334.73±6.62***	390.58±18.79*
T_4(AF-TQ)	310.03±24.49	306.56±23.85	259.48±9.69**	230.39±8.78**	322.42±7.09**	334.05±3.79*
T_5(TQ-AF)	170.42±0.72***	159.43±1.29***	201.38±9.30***	245.38±2.58***	279.38±3.21***	274.52±2.58***

Glutathione Peroxidase (GPx) Activity

The normal value of the GPx activity was between (909.93±5.63)and (983.70±1.41).

It was found that administration of *Aspergillus fumigates* either after, before, without or combined with thymoquinone induced a decrease in the GPx activity at all groups in the 4th week post-injection. On the other hand, administration of thymoquinone either after, before, without or combined with *Aspergillus fumigates* induced an increase in the GPx activity at all groups all over the experimental period except at group (T_3) in the 3rd and 4th weeks post-injection. A very highly significant increase was noticed at groups (T_4) and (T_5) (Table 5).

Table 5: Effect of thymoquinone on Glutathione Peroxidase (GPx) activity in *Aspergillus fumigatus* sensitized albino rats (mU/mL).

Time/Week / Groups	W1	W2	W3	W4	W5	W6
N_0	972.52±1.14	983.70±1.41	940.40±29.85	960.08±2.81	931.88±3.70	909.93±5.63
C_1(PBS)	1468.71±1.09	1558.22±1.12	1583.51±1.12	1640.89±1.69	1473.60±2.81	1556.86±0.78
C_2(TW20%)	1382.09±4.57	1378.27±1.69	1375.36±1.12	1392.47±3.14	1409.40±5.05	1465.82±2.81
C_3(PBS+TW20%)	1451.22±2.25	1433.90±12.92	1470.45±8.19	1507.64±3.37	1489.16±2.81	1482.35±6.74
C_4(PBS-TW20%)	1485.26±2.85	1587.03±2.25	1516.21±2.24	1676.33±2.81	1327.70±2.81	1345.20±3.93
C_5(TW20%-PBS)	1400.16±2.53	1442.47±0.56	1357.85±4.50	1484.29±2.25	1439.55±2.25	1443.25±1.01
T_1(AF)	1438.73±0.61***	1388.00±1.69***	1509.59±5.61***	1518.34±1.68***	1448.48±3.74**	1486.24±4.49***
T_2(TQ)	1561.13±2.81***	1577.62±1.51***	1525.14±2.25***	1563.08±2.81***	1442.47±2.81**	1492.86±4.49**
T_3(AF+TQ)	1472.62±2.25**	1469.88±1.37	1457.05±1.12	1417.18±5.05***	1502.78±2.81*	1489.26±7.36
T_4(AF-TQ)	1426.38±7.10**	1320.89±10.11***	1331.58±3.93***	1429.43±3.49***	1559.79±2.25***	1551.04±6.18***
T_5(TQ-AF)	1545.43±3.03***	1588.00±1.69***	1465.84±3.95***	1366.60±2.81***	1355.90±4.49***	1364.66±3.93***

Glutathione-S-Transferase (GST) Activity

The normal value of the GST activity was between (2331.52±1.14) and (2390.20±5.72).

Table (6) revealed a decrease in the GST activityall over the experimental period as a result of injection of *Aspergillus fumigatus* either after, before, or without thymoquinone except at group (T_1) in the 2nd week and at group (T_5) in the 3rd week post-injection while injection of thymoquinone either after, before, without or combined with *Aspergillus fumigates* induced a very highly significant increase in the GST activity all over the experimental period.

Table 6: Effect of thymoquinone on Glutathione-S-Transferase (GST) activity in *Aspergillus fumigatus* sensitized albino rats (U/L).

Time/Week / Groups	W1	W2	W3	W4	W5	W6
N_0	2380.32±3.45	2390.20±5.72	2331.52±1.14	2355.93±4.04	2343.72±2.29	2366.65±0.82
C_1(PBS)	2364.87±7.47	2353.64±979	2291.75±6.34	2446.44±5.75	2471.74±3.45	2460.05±2.87
C_2(TW20%)	2187.74±1.18	2196.18±2.26	2075.26±3.42	2139.94±6.36	2204.61±0.46	2002.14±2.88
C_3(PBS+TW20%)	2044.29±2.88	2196.17±6.92	2190.52±1.72	2184.86±2.30	2165.24±4.06	2066.82±5.76
C_4(PBS-TW20%)	2322.77±9.20	2337.82±23.14	2303.41±8.75	2313.38±7.39	1889.66±6.29	1926.22±4.06
C_5(TW20%-PBS)	2075.53±0.74	2004.60±0.99	2156.47±9.35	2193.24±2.21	2179.19±4.09	2109.00±2.31
T_1(AF)	2350.40±3.46	2502.68±8.67***	2290.23±5.79	2291.79±2.88***	2387.36±6.32***	2333.96±7.51***
T_2(TQ)	2572.88±2.86***	2560.85±0.55***	2558.81±6.32***	2533.58±3.43***	2536.41±2.28***	2561.73±1.16***
T_3(AF+TQ)	2886.52±5.77***	2809.52±3.76***	2963.85±2.34***	2904.72±1.75***	2980.72±6.37***	3124.13±2.87***
T_4(AF-TQ)	2278.66±5.26*	2213.91±5.18**	2269.45±0.59*	2240.58±7.85**	2958.29±22.13***	3132.57±5.87***
T_5(TQ-AF)	2566.18±2.83***	2588.54±2.21***	2198.67±4.86*	2141.17±1.77	2119.36±2.84***	2105.87±1.72

Lipid Peroxide (malondialdehyde)

The normal value of MDA level was between (33.28±1.17) and (33.52±5.25).

Through the intranasal injection of *Aspergillus fumigates* either after, before, or without thymoquinone, MDA level was increased all over the experimental period exceptat group (T_5) in the 3rd week post-injection where the lipid peroxide level decreased. On the other hand, through the i.p injection of thymoquinone either after, before, without or combined with *Aspergillus fumigatus*, MDA level decreased all over the experimental period (Table 7).

Glutathione Reduced (GSH) Concentration

The normal value of GSH concentration was between (45.26±2.92) and (45.40±3.50).

It was found that administration of *Aspergillus fumigatus* either after, before, or without thymoquinone induced a decrease in GSH concentration all over the experimental period except at group (T_5) in the 3rd week post-injection. On the other hand, administration of thymoquinone either after, before, without or combined with *Aspergillus fumigates* induced an increase in GSH concentration all over the experimental period (Table 8).

Table 7: Effect of thymoquinone on serum lipid peroxide level (MDA) in *Aspergillus fumigatus* sensitized albino rats (nmol/ml).

Time/Week / Groups	W1	W2	W3	W4	W5	W6
N_0	33.34±3.50	33.52±5.25	33.28±1.17	33.31±4.08	33.43±2.33	33.43±0.58
C_1(PBS)	44.69±10.50	44.42±9.91	44.62±6.15	44.48±5.83	44.63±3.50	44.93±2.92
C_2(TW20%)	48.30±1.17	44.93±2.33	46.63±3.50	45.82±6.41	45.34±0.58	45.88±2.92
C_3(PBS+TW20%)	36.33±2.92	37.60±6.73	37.07±1.75	43.28±2.33	40.51±4.08	40.36±5.83
C_4(PBS-TW20%)	42.63±4.08	44.78±3.50	44.06±6.39	44.36±0.58	46.69±5.83	47.73±5.25
C_5(TW20%-PBS)	49.19±1.75	48.66±2.92	44.18±4.08	43.73±4.67	43.88±2.33	44.78±3.50
T_1(AF)	48.36±3.50	48.81±8.75	50.45±5.83	51.64±2.66	52.24±6.41	52.51±7.58
T_2(TQ)	46.78±2.92	43.07±0.58	45.08±6.40	44.00±3.46	43.55±2.33	43.13±1.17
T_3(AF+TQ)	33.88±5.83	33.85±4.08	34.69±2.33	37.31±2.92	36.45±6.41	35.58±2.92
T_4(AF-TQ)	45.46±5.25	48.64±5.56	51.64±0.58	52.24±6.41	44.30±3.50	41.22±4.08
T_5(TQ-AF)	44.63±3.50	43.88±2.33	43.85±4.67	51.64±4.08	52.39±2.92	53.13±1.75

Table 8: Effect of thymoquinone on Glutathione Reduced (GSH) concentration in *Aspergillus fumigatus* sensitized albino rats (mg/dL).

Time/Week / Groups	W1	W2	W3	W4	W5	W6
N_0	45.33±1.17	45.30±1.75	45.40±3.50	45.26±2.92	45.28±0.58	45.34±1.75
C_1(PBS)	56.19±0.58	57.39±1.75	53.33±5.83	53.66±1.75	60.33±3.50	56.63±4.94
C_2(TW20%)	54.79±4.67	56.19±1.75	53.39±1.17	56.46±2.92	61.33±5.25	58.90±2.92
C_3(PBS+TW20%)	51.86±1.17	52.65±2.04	52.13±3.50	48.53±1.17	49.46±1.75	48.64±2.62
C_4(PBS-TW20%)	54.04±4.07	55.86±2.92	55.33±2.33	52.19±2.92	57.19±2.33	58.73±1.75
C_5(TW20%-PBS)	53.00±0.58	54.33±1.17	56.86±2.33	60.33±4.08	59.66±1.75	59.64±2.62
T_1(AF)	49.60±1.17**	50.93±1.17*	48.40±1.17	47.13±1.75	51.64±2.62	49.56±0.58
T_2(TQ)	61.26±2.33	61.39±2.33	61.13±1.75*	63.90±4.08	66.66±2.92	61.19±4.08
T_3(AF+TQ)	56.65±2.04	56.26±1.17	57.09±1.16	57.93±3.50	59.66±2.92*	58.73±1.75*
T_4(AF-TQ)	49.83±1.75	52.73±2.33	46.93±2.92	52.06±2.33	66.66±2.92	65.93±4.08
T_5(TQ-AF)	61.43±2.92	60.46±1.75*	57.39±4.08	58.98±2.32	56.06±1.17	59.06±0.58

Glutathione Reductase (GR) Activity

The normal value of GR activity was between (430.35±5.83) and (530.51±0.58).

Table (9) revealed a very highly significant decrease in GR activityall over the experimental period as a result of injection of *Aspergillus fumigatus* either after, before, or without thymoquinone except at group (T_5) in the 3rd and 4th weeks post-injection while injection of thymoquinone either after, before, without or combined with *Aspergillus fumigatus s*induced an increase in GR activity allover the experimental period.

Table 9: Effect of thymoquinone on Glutathione Reductase (GR) activity in *Aspergillus fumigatus* sensitized albino rats (U/L).

Time/Week / Groups	W1	W2	W3	W4	W5	W6
N_0	430.35±5.83	480.43±4.08	505.47±3.50	477.80±1.17	530.51±0.58	450.13±2.33
C_1(PBS)	720.13±7.58	630.93±9.87	724.15±6.41	482.28±5.83	562.66±3.50	556.63±2.92
C_2(TW20%)	510.41±1.17	454.48±2.63	538.55±3.50	580.75±6.41	622.95±0.58	610.03±2.91
C_3(PBS+TW20%)	465.74±2.92	360.82±6.99	209.98±1.75	413.28±2.33	420.66±4.08	430.35±6.07
C_4(PBS-TW20%)	630.51±9.33	656.56±8.16	689.62±5.83	518.49±5.25	522.90±2.92	518.45±8.75
C_5(TW20%-PBS)	550.60±3.50	510.41±2.92	725.06±1.75	741.62±4.67	650.23±2.33	674.03±2.91
T_1(AF)	502.38±3.50***	454.15±8.75***	289.37±5.83***	385.82±2.92***	293.39±6.41***	419.95±7.55***
T_2(TQ)	1740.23±2.92***	1572.16±0.58***	1905.34±6.65***	1274.03±3.49***	1481.49±2.33***	1892.95±1.17***
T_3(AF+TQ)	622.95±5.83***	669.75±4.08***	656.05±2.33***	682.28±1.75***	520.64±6.41***	571.80±2.92***
T_4(AF-TQ)	506.39±5.25***	490.32±5.25***	333.58±0.58***	373.77±8.16***	570.70±11.66*	610.89±5.83**
T_5(TQ-AF)	1206.75±2.92***	1274.75±2.33***	1082.28±4.67***	853.20±1.75***	541.62±2.92***	526.03±3.48***

N_0= Free injected, PBS= Phosphate buffered saline, TW20%= TWEEN20%, AF= Aspergillus fumigatus, TQ= Thymoquinone, Values are expressed as means ± SE

** P‹0.05 ** P‹0.01 *** P‹0.001 ( ) P›0.05*

Discussion

Oxidative stress plays an important role in the pathogenesis of airway diseases such as asthma which is a chronic inflammatory disorder of the airways involving a complex interaction of cells and mediators, most of which result in increased reactive oxygen and nitrogen species (ROS and RNS, respectively) in the airways (Dweik *et al*., 2001 and Kumaraguruparan *et al*., 2002). ROS are essential components of the defensive

mechanism against fungus infection and are involved in many of the complex interactions between the invading microorganisms and its host (Miller &Britigan, 1997).

Thymoquinone is the abundant *Nigella sativa* essential oil compound that is known to be the active principle responsible for many of the seed's antioxidant and anti-inflammatory effects (Gali-Muhtasib *et al*., 2008). In this regard, earlier studies have reported that TQ prevents oxidative damage induced by a variety of free radical generating agents (Mohamed *et al.*, 2005).

In this study, it was found that administration of *Aspergillus fumigates* either before, without or combined with thymoquinone increased the serum total protein concentration all over the experimental period exceptat groups (T_3) in the 4th week and (T_5) in the 3^{rd,} ,4th, and 5th weeks post-injection. This increase may be due to increased airway secretion of surfactant proteins A and D that was stimulated by allergic inflammation. The increased levels of surfactant proteins A and D may play a protective role in both host defense and in an allergic inflammation in the pathogenesis of bronchial asthma (Cheng *et al*., 2000). While, the decrease observed at group (T_3) in the 4th week post-injection may be due to indirect effect of free radicals through DNA and RNA damage whereas, free radicals are a major source for DNA damage, which can cause strand breaks and base alteration in the DNA (Trivedi *et al*., 2008). On the other hand, administration of thymoquinone either after or before *Aspergillus fumigatus* increased the serum total protein concentration. This increase could be attributed to the strong antioxidant potential of thymoquinone in the suppression of oxidative stress (Abdel-Wahab, 2013).

Through the whole experiment, SOD and CAT activities were decreased at all treated groups exceptat group (T_4) in the 2nd week post-injection where the SOD activity was increased. The inhibition of SOD and CAT activities could presumably be explained as a consequence of less availability of the substrates for these enzymes (superoxide radical or free radicals) due to the superoxide radical scavenging effect of thymoquinone (Nagi& Mansour, 2000).This finding is in harmony with the study which demonstrated that treatment with antioxidants resulted in reduction in antioxidant enzyme activities(Patra *et al*., 2001) or may be due to enhanced production of oxygen radicals by inflammatory cells (Comhair *et al*., 2000) . Moreover, SOD and CAT activities in the lung are related to airway hyper reactivity and airflow limitation (Comhair *et al*., 2005). This result agrees with the study hypothesized that asthmatic individuals with higher levels of oxidative stress may have greater loss of SOD and CAT activities, which would be reflected systemically in loss of circulating SOD and CAT activities (Comhair & Ricci, 2005). While, an increase recorded by group (T_4) in the 2nd week post-injection may be due to activated peripheral blood monocytes of atopic individuals which produce superoxide when IgE binds to membrane receptors (Demoly *et al*., 1994) and serum eosinophil cationic protein, a biomarker of eosinophil activation, is increased with atopy and asthma severity indices (Joseph-Bowen *et al*., 2004).

The decrease in the Glutathione Peroxidase (GPx) activity after administration of*Aspergillus fumigatus* either after, before, without or with thymoquinone at all groups in the 4th week post-injection may be due to an increase in generation of oxidants by peripheral blood leukocytes when exposed to AFs (Petrone *et al*., 1980) which was found to play a role in inactivating red cell GPx (Blum & Fridovich, 1985). On the other hand, administration of thymoquinone either after, before, without or combined with *Aspergillus fumigatus* induced an increase in the GPx activity at all groups all over the experimental period except at group (T_3) in the 3rd and 4th weeks post-injection. This increase may be due to the strong antioxidant potential of thymoquinone (Houghton *et al*., 1995) where Woo *et al*. (2012) reported that thymoquinone can scavenge free radical and preserve the activity of various antioxidant enzymes such as GPx. Previous studies have also shown that thymoquinone could upregulate the GPx gene with the consequent elevation of hepatic GPx level to overcome oxidative stress (Ismail *et al*., 2010& Nagi and Almakki, 2009).

It was found that administration of *Aspergillus fumigatus* either after, before, or without thymoquinone decrease the Glutathione-S-Transferase (GST) activity all over the experimental period except at group (T_1) in the 2nd week and at group (T_5) in the 3rd week post-injection. This result may be due to the severity of asthma where antioxidant deficiency in the lung are related to severity of airflow limitation and hyperreactivity (Comhair *et al*., 2005) orthe impaired antioxidant defense in the asthmatic airway (Comhair *et al*., 2000). While, an increase was observed at groups (T_1) and (T_5) in the 2nd and 3rd weeks post-injection thatmay be due to GSTP1 which is the major isoform in erythrocytes and platelets whereas, the GSTP1 phenotype may play an indirect role in asthma via its effect on platelet function by increasing platelet aggregation in asthma (Mak *et al*., 2007). On the other hand, injection of thymoquinoneeither after, before, without or with *Aspergillus fumigatus* induced a very highly significant increase in the GST activity all over the experimental period. This increase may be due to the upregulation action of thymoquinone on the GST gene with the consequent elevation of hepatic GST level to overcome the induced oxidative stress (Ismail *et al*., 2010 & Nagi and Almakki, 2009).

The results demonstrated an increase in the lipid peroxide (MDA) level allover the experimental period except at group (T_5) in the 3rd week post-injection through the intranasal injection of *Aspergillus fumigatus* either after, before, or without thymoquinone and this result may be due to an allergic response where, eosinophils migrate, into the tissue, and readily degranulate, releasing cytotoxic products such as granule proteins and ROS (Rahman *et al*., 1996). Lipid peroxides can be formed in the presence of oxygen free radicals

(Walsh, 1994) and earlier reports showed increase in the oxidative stress in asthma including the presence of increased lipid peroxidation products in plasma consistent with enhanced production of ROS in blood monocytes, neutrophils and eosinophils (Vachier *et al*., 1992). On the other hand, MDA level was decreased all over the experimental period after administration of thymoquinone either after, before, without or with *Aspergillus fumigatus* and this decrease may be due to the marked anti-oxidative stress effect of thymoquinone which is suggested to be through inhibition of lipid peroxidation (Nagi *et al.*, 1999). Also, this inhibition in MDA level may be the initial event in the mechanism by which thymoquinone ameliorates hepatotoxicity (Nagi *et al*., 1999).

Administration of *Aspergillus fumigatus* either after, before, or withoutthymoquinone induced a decrease in Glutathione Reduced (GSH) and Glutathione reductase (GR) concentrations all over the experimental period except at group (T_5) in the 3rd week post-injection. This decrease may be related to the oxidative stress where, the induction of GR activities is a potential biochemical marker of oxidative stress and decreased GR activity may lead to GSH depletion where the role of GR is to sustain the cytosolic concentration of GSH (Sun *et al*., 2006). On the other hand, administration of thymoquinone either after, before, without or with *Aspergillus fumigatus* induced an increase in GSH and GR concentrations all over the experimental period. This increase may be due to the effect of thymoquinone on phase II detoxification system. GSH and its related enzymes, namely GST, GR and GPx are key players in the detoxification of reactive metabolites and the prevention of cellular oxidative damage (Elbarbry and Alcorn, 2009). Thymoquinone elevated GR; an enzyme that maintains glutathione in its reduced form that detoxifies reactive metabolites. (Hayes *et al*., 2005).

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