

Thyme and Thymol Effects on Induced Bronchial Asthma in Mice

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Abstract: Asthma is very common in Saudi Arabia. It is characterized by sporadic occurrence of inflammation and swelling of the inner lining of the lung with an increase in the secretion of sticky mucus, cough and muscle contraction of the chest. Under normal circumstances, the defense system of the body has the balance between the production of oxidizing substances and antioxidant. However, under increased exposure to oxidizing materials the body is unable to cope and this results in an oxidative stress causing many ailment including asthma. The main aim of the study was to evaluate the state of oxidative stress in bronchial asthma induced mice as well as a comparative estimation of the antioxidant effect of thyme and thymol. The study included the induction of bronchial asthma using ova albumin followed by the treatment with thyme and thymol. Estimation of two antioxidant enzymes, superoxide dismutase and glutathione peroxidase. In addition the concentration of lipid peroxidation products of binary malondialdehyde and 8-isoprostaglandin F_{2α} were also investigated. Total protein, carbonyl protein and hemoglobin level were also assessed. The results of the biochemical indicators obtained from groups of mice treated were compared the results of asthmatics group. The results showed that samples of asthmatics group had high rates of oxidative stress, accompanied by a major imbalance in the amount of antioxidants. In addition, high levels of lipid peroxidation products and carbonyl protein, was also associated with a reduction in the rates of total protein and blood content of hemoglobin. The results also showed groups of mice treated with thyme and thymol showed a significant improvement in the level of all the studied parameters. It was accompanied by apparent decline in the rates of free radicals and oxidative agent and lipid peroxidation products compared to the control groups. In conclusion, the study indicates that thyme and thymol increased the rates of antioxidants in the body, and the ability to get rid of oxidative agent and free radicals that are generated inside the body, or due pollution environment. Hence, this study confirms the potential effect of both thyme and thymol as possible means to treat asthma.

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1. Introduction

Bronchial asthma is a complex syndrome characterized by airway hyperresponsiveness (AHR) and reversible airflow obstruction associated with airway inflammation and remodeling and occasional high serum level of IgE (Cohn *et al.*, 2004). Histologically, there are infiltrates of eosinophils, degranulated mast cells, subbasement membrane thickening, hyperplasia and hypertrophy of bronchial smooth muscle, and hyperplasia of airway goblet cells (Elias *et al.*, 2003).

The inflammatory cells infiltrating the airways produce several mediators that modulate the inflammatory response. These include a range of toxic reactive oxygen species (ROS), such as superoxide radical, hydrogen peroxide, hypochlorous acid, and hydroxyl radical (Chanez *et al.*, 1990 & Vachier *et al.*, 1992). The ROS have been associated with many pathophysiologic changes that are relevant in asthma, such as increased lipid peroxidation, increased airway reactivity and secretions, increased production of chemoattractants, and increased vascular permeability (Barnes,1990).

The lung and blood are endowed with several antioxidants, to counter the oxidant-mediated toxicity, including glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase, glutathione, vitamin E, and vitamin C (Toth *et al.*, 1984 & Heffner and Repine, 1991). There is increased oxidative stress in asthma, as shown by an increased protein carbonyls and production of lipid peroxidation products including 8-iso-prostaglandin F_{2α} (8-iso-PGF_{2α}) and malondialdehyde (MDA) in plasma (Wood *et al.*, 2003), enhanced generation of ROS by blood monocytes, neutrophils, and eosinophils (Rahman,1996), increased oxidized glutathione in bronchoalveolar lavage (BAL) fluid (Kelly *et al.*, 1999), and increased production of nitric oxide (NO) in exhaled air (Kharitinov *et al.*, 1994). On the other hand, changes in antioxidant defenses have been reported, including decreased GSH-Px in whole blood, plasma, and platelets; a deficiency of selenium (Picado *et al.*, 2001), decreased protein sulfhydryls and total antioxidant capacity in plasma (Rahman,1996), increased SOD activity in BAL cells (Smith *et al.*, 1997), and

decreased vitamin C and vitamin E concentration in BAL fluid (Kelly *et al.*, 1999).

Many studies have been shown that the presence of natural antioxidants from various aromatic and medicinal plants is closely related to the reduction of chronic diseases such as DNA damage, mutagenesis, and carcinogenesis (Reddy *et al.*, 2003).

Overproduction of free radicals in organisms and lipid peroxidation in cell membranes has been implicated in various pathophysiological disorders, including cardiovascular diseases, mutagenesis, diabetes, ischemia-reperfusion injury, coronary atherosclerosis, Alzheimer's disease, and cancerogenesis, as well as the aging process (Smith *et al.*, 2002).

Since ancient times, aromatic herbs and spices have been added to different types of food to improve the flavor and organoleptic properties. Also, they have great potential in the emerging nutritious industry, because these materials are often considered as food and medicines, as well, and are used in prevention and curative treatments throughout the world (Diplock *et al.*, 1998). Besides, many essential oils and isolated compounds were recently qualified as very strong natural antioxidants (Mimica-Dukic *et al.*, 2004 & Bozin *et al.*, 2006) and proposed as potential substitutes for synthetic antioxidants.

The thyme plant is a permanent, herbaceous shrub belonging to the family Lamiaceae. It is commonly grown wild throughout the Mediterranean region "Spain, France and Italy; and in almost every Caribbean country. The herb was used by the Greeks as an incense in their temples and by the Romans in cooking and as a source of honey. It can be used whole or ground for seasoning foods (Balladin & Headley, 1999).

It has long been used as a source of the essential oil (thyme oil) and other constituents (e.g. thymol, flavanoid, caffeic acid and labiatic acid) derived from the different parts of the plant. The pharmacological properties of the plant and of its different extracts, in particular the essential oils, has been thoroughly studied and afforded the many industrial (mainly as food additive) and medical applications of the plant (Caillet *et al.*, 2007). In addition to their numerous traditional uses, the plant (herb) and its essential oil have found diverse applications in pharmacy and medicine. The oil was reported to have antimicrobial (bacteria and fungi, carminative and expectorant activities, most of which are mediated by thymol and carvacrol, as the phenolic components (Hudaib *et al.*, 2002).

2. Materials and methods

Male Wistar albino mice weighing between 60-120 gm were acquired from the experimental animal

house- Faculty of Pharmacy- King Saud University- Riyadh. Mice were housed individually in standard cages and were maintained on standard pellet diet and tap water and kept at $30 \pm 3^\circ\text{C}$ temperature, 50–60% humidity, and a 12 h light-dark cycle. This standard diet consists of 20% crowd protein, 3% fat, 0.8 % calcium, 0.6 % phosphorous, 0.5 % sodium chloride, 5.5 % fibers as well as the other trace elements added such as; cobalt, copper, iodide, iron, manganese and zinc. The acclimatization conditions last for two weeks before the commencement of the experiment. All animals received professional human care in compliance with the guidelines of the Ethical Committee of the University.

Experimental design:

Sixty male Wistar albino Mice were randomly divided into four groups, fifteen rats each: one group as a control; was administered orally with sterile distilled water. Second group was administered 1.8 aluminum hydroxide. The other Mice were induced for bronchial asthma by immunization with 20 μg m ova albumin (OVA) adsorbed to 1.8 mg aluminum hydroxide/ Kg body weight according to Russo *et al.*, 1998. 0.5 ml of OVA were injected intraperitoneally once. At the fourteenth day after immunization, the Mice were challenged by exposure to an aerosol of OVA for 20 minutes at a concentration of 25 mg/ml in 0.9 % saline generated by an ultrasonic nebulizer (ICEL US-800, SP. Br). Finally, Mice were received OVA in aerosol form on alternate days for 10 days. After the end of the induction, blood samples were withdrawn from the optical vein and tested for the oxidants and the antioxidants evaluated in this study to ensure the induction of bronchial asthma. Mice were divided into two groups; one was administered orally 0.06 g/Kg body weight of thyme extract daily for two weeks according to Youdium & Deans, 1999. The other group was administered 0.7 μg /ml/Kg body weight of thymol dissolved in drinking water daily for two weeks according to Braga *et al.*, 2006.

By the end of day fourteen after treatment, each rat was made to fast for 24 hours and then perfused under ethyl ether (30 mg/kg, 100 mg/mL) and Xylazine (3 mg/kg, 100 mg/mL) anesthesia. Blood samples were withdrawn from the optical vein into polypropylene tubes; with and without anticoagulants. Samples were centrifuged at 3000 rpm for separation of sera that were stored at -20°C until assayed. Whole blood samples were assayed immediately.

Biochemical parameters:

Serum samples were assayed for total protein according to Josephson & Gyllensward, 1957, carbonyl protein according to Levine *et al.*, 1990, malondialdehyde according to Vento, 2000, 8-

isoprostaglandin according to Lawson & Maxey, 1996, hydrogen peroxide according to McNamara & Augusteyn, 1984 and nitric oxide according to Miles, 1996 & Maeda, 2004. Whole blood samples were assayed for hemoglobin according to Van Kampen & Zijlstra, 1961, glutathione peroxidase according to Kraus & Ganther, 1980 and super oxide dismutase according to Suttle, 1986.

Statistical analysis:

The data analysis was carried out using the statistical package for social science (SPSS software version 16, Chicago, Illinois). All numeric values were expressed as mean \pm SE. Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by Post Hoc LSD test using Bonferroni multiple comparisons. Pearson's correlation test was used for correlating variables. For all tests a probability value < 0.05 was considered significant.

3. Results:

Data of the present study revealed a state of airway inflammation and bronchial asthma as represented in table (1). The group of mice administered aluminum hydroxide showed no statistical significant difference in the evaluated parameters when compared to ova albumin administered counter parts.

Symptoms of bronchial asthma has been proved biochemically in groups administered ova albumin by the statistical significant increase in nitric oxide, malondialdehyde, isoprostane, carbonyl group and hydrogen peroxide serum levels as compared to their normal counter parts ($P < 0.001$). Moreover, such result was confirmed by a statistical decrease in both hemoglobin and whole blood superoxide dismutase activity ($P < 0.001$).

Table (1): Investigated parameters in the studied groups.

	Control	Ova	Thyme	Thymol
Hemoglobin	15.25 \pm 0.17	10.9 \pm 0.22 ^{a**}	12.67 \pm 0.39 ^{a**,b**}	12.82 \pm 0.76 ^{a**,b**}
Nitric oxide	54.6 \pm 2.9	115.4 \pm 4.6 ^{a**}	58.4 \pm 7.8 ^{b**}	75.5 \pm 4.9 ^{a*,b**}
Hydrogen peroxide	83.6 \pm 1.67	137.4 \pm 5.2 ^{a**}	95.5 \pm 1.6 ^{b**}	116 \pm 9.1 ^{a**,b**,c*}
Malondialdehyde	2.4 \pm 0.21	11.8 \pm 0.82 ^{a**}	6.45 \pm 0.36 ^{a**,b**}	6.5 \pm 0.3 ^{a**,b**}
Isoprostane	230.2 \pm 19.1	767.7 \pm 14.2 ^{a**}	499 \pm 15.9 ^{a**,b**}	531 \pm 34.8 ^{a**,b**}
Carbonyl group	0.51 \pm 0.019	0.96 \pm 0.07 ^{a**}	0.57 \pm 0.02 ^{b**}	0.59 \pm 0.017 ^{b**}
Glutathione peroxidase	137.8 \pm 8.1	106.9 \pm 4.5	177 \pm 5.5	190 \pm 4.9 ^{a**,b**}
Superoxide dismutase	345 \pm 11.5	195.8 \pm 9.3 ^{a**}	316 \pm 12.9 ^{b**}	299 \pm 11.6 ^{a*,b**}

Data are represented as mean \pm SE. a comparison vs control, b vs ova, c vs thyme. * $P < 0.05$; ** $P < 0.001$

Wheezing episodes were diminished after the treatment with both thyme and thymol. Both treatments revealed a significant improvement in the state of oxidative stress as well as a significant elevation in hemoglobin level ($P < 0.001$) as represented in figure (1).

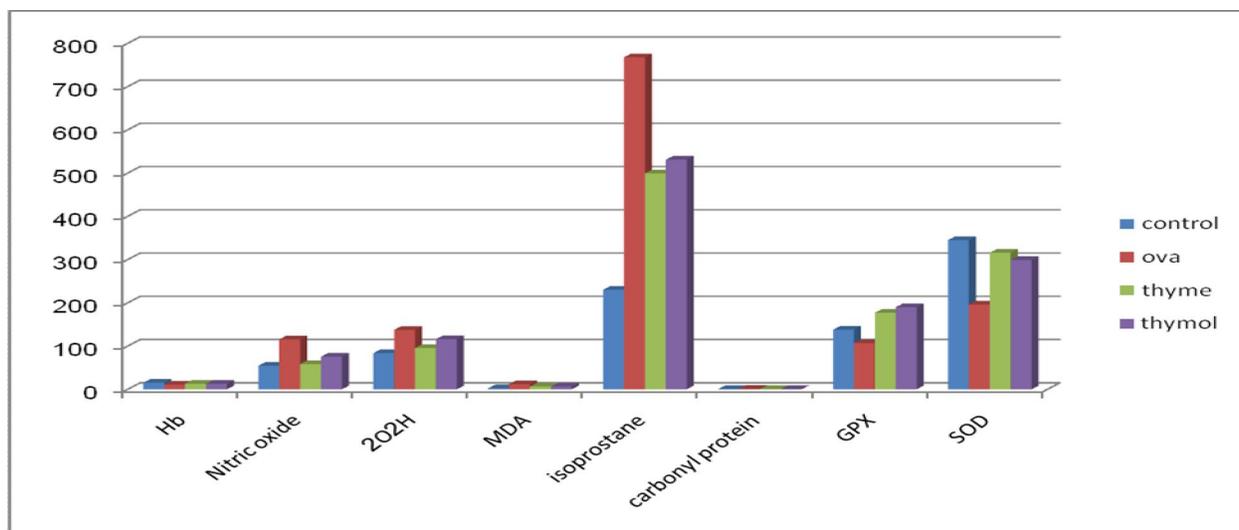


Figure (1): Comparison of the studied parameters in all groups

Oxidative stress as revealed by parameters of both oxidants and antioxidants showed the better improvement in group administered thyme. This was

clarified by the absence of statistical significant difference in most of these parameters as compared to those of the control counterparts.

The correlation between the investigated oxidant and antioxidant status using Pearson's correlation is represented in table (2). It reveals a highly statistically significant negative correlation between superoxide dismutase as well as hemoglobin with the evaluated oxidant parameters ($p < 0.001$). Besides, a highly statistically significant positive correlation within the studied oxidants was also shown.

Table (2): Pearson's correlation coefficient in the studied groups

	Superoxide dismutase	Malodialdehyde	Isoprostane	Carbonyl group	H ₂ O ₂
Hemoglobin	NS	-0.688**	-0.817**	-0.608**	-0.563*
Nitric oxide	-0.678**	0.716**	0.651**	0.551**	0.619**
Isoprostane			-----	0.618**	0.646**
Malodialdehyde		-----	0.758**	0.659**	0.787**
Glutathione peroxidase	NS	NS	NS	-0.395*	NS
Superoxide dismutase	-----	-0.728**	-0.409*	-0.671**	-0.607**

* $P < 0.05$; ** $P < 0.001$

In this study, we demonstrated the presence of early airway hyperresponsiveness or bronchoconstrictive responses induced by ovalbumin. Our data clearly showed that the increase in airway hyperresponsiveness caused by ovalbumin is improved upon treatment with both thyme and thymol extracts.

Ovalbumin has been shown previously to induce asthma in mice (Daheshia *et al.*, 2002). Many genes observed in this study have been previously shown to be associated with asthma, eosinophilia, airway hyperreactivity, or epithelial cell metaplasia (Follettie *et al.*, 2006 & Rolph *et al.*, 2006).

Exposure to different stimuli results in the generation of reactive oxygen species (ROS) in the airway epithelial cells, which produce inflammatory cytokines and chemokines and express adhesion molecules on their cell surface and cause airway inflammation, which involves narrowing of airways, secretion of large amounts of mucus, and infiltration of inflammatory cells (Holgate, 2008). This was clarified in the present work as indicated by elevated levels of nitric oxide, carbonyl protein, hydrogen peroxide, malondialdehyde and 8-isoprostane.

Increasing evidences suggest that ROS play an important role in the pathogenesis of airway inflammation during asthma (Sheppard, 2009). ROS play an important role in the pathogenesis of airway inflammation during asthma by disturbing the cellular redox homeostasis.

With growing understanding of the role of ROS in mediating the airway inflammation, various studies have suggested the use of antioxidants to treat such inflammation (Kirkham & Rahman, 2006). Although the antioxidant capacity of airway epithelial cells is excellent, upon repeated and continued

4. Discussion:

Recently, studies on the effect of antioxidant foods and health functional foods on the prevention and treatment of various chronic diseases have been conducted. Such substances have been reported to possess antioxidant function and normalize compromised antioxidant systems by the oxidative stress caused by free radicals (Lee *et al.*, 2011).

exposure to allergens, the antioxidant capacity decreases. This further augments the ROS generation and inflammation. Therefore, antioxidant(s) or the compounds that could block the inflammatory signals and/or the transcription of inflammatory markers could be excellent drugs to treat airway inflammation. Antioxidant levels were reduced in ovaalbumin treated group that was elevated upon oral administration of both thyme and thymol, however, thyme showed the best improvement.

There has been a great deal of interest in the role of complementary and alternative medicines for the treatment of various acute and chronic diseases (Hussein *et al.*, 2010). Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness (Al-Jamal & Alqadi, 2011). An imbalance between oxidative stress and antioxidative capacity may play an important role in the development and progression of bronchial asthma (BA) and chronic obstructive pulmonary disease (COPD) Gumral *et al.*, 2009.

Oxidative stress is involved in asthma. Carbonylated proteins (68 kDa and 53 kDa) were elevated in asthmatics when compared to controls and the 68-kDa carbonylated protein was significantly correlated with sputum eosinophilia (Nagai *et al.*, 2008).

In the present work, we have shown that carbonyl group level was elevated in bronchial asthma induced group. Similar findings were observed in mice after chronic exposure to ozone for 6 weeks. Antibodies against carbonyl-modified protein were elevated and splenocytes isolated from ozone-exposed mice became activated in response to stimulation with carbonyl-modified protein. This was accompanied by a greater antigen-presenting cell

activation (both macrophages and dendritic cells) in murine lungs as demonstrated by the increased expression of the activation markers CD80, CD86, and CD54 on these cells (Kirkham *et al.*, 2011).

In the present work we investigated whether the formation of F(2)-isoprostanes was associated with increased ovalbumin (OVA)-induced airway inflammation. Jonasson and his colleagues, 2009 showed an accumulation of F(2)-isoprostanes in acute airway inflammation and a markedly increased tissue damage caused by oxidative stress in an ongoing inflammation.

Inflammatory cells (such as activated eosinophils, neutrophils, monocytes, and macrophages) and resident cells (such as epithelial and smooth muscle cells) can generate reactive oxygen species (ROS). The sources of these species include primarily nicotinamide adenine dinucleotide phosphate (NADP) oxidase-dependent complex, the cytosolic xanthine oxidase system, and the mitochondrial respiratory chain. Oxygen spontaneously or enzymatically dismutates to hydrogen peroxide (H₂O₂). Both O₂ and H₂O interact with iron and other metal ions and form OH⁻ in biological systems. Eosinophils, neutrophils, and monocytes contain peroxidases that catalyze the interaction between H₂O and halides leading to the formation of hypohalides, such as HOCl. In addition, superoxide anion may also react with nitric oxide (NO) to form peroxynitrite (ONOO⁻), a potent ROS (Sahiner *et al.*, 2011).

To our knowledge, thyme and thymol were not previously studied as a bronchial asthma treatment.

In conclusion, this pilot study has demonstrated that asthma is associated with a strong oxidant stress that is a result of both increased oxidant forces and decreased antioxidant capacity. Even though modulation of this system offers great promise in the treatment of inflammatory diseases, such as asthma. We recognized that one of the important limitations of this study is the low numbers of subjects screened in each group. Therefore, it would be important to confirm our findings here in larger cohorts of control subjects and patients as well.

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