

Modulatory Effect of Parsley and Vitamin C in Ovalbumin Induced 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 ailments 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 Parsley and Vitamin C. The study included the induction of bronchial asthma using ova albumin followed by the treatment with parsley and vitamin C. 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 parsley and ascorbic acid showed a significant improvement in the level of antioxidant enzyme, total proteins and hemoglobin. It was accompanied by apparent decline in the rates of free radicals and oxidative agent, carbonyl protein and lipid peroxidation products compared to the control groups. In conclusion, the study indicates that Parsley 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 parsley and ascorbic acid as possible means to treat asthma.

Key words: Bronchial asthma- Parsley- Vitamin C- Oxidant - Antioxidant

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 and 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).

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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). Parsley (*Petroselinum crispum*) is native to Europe and Western Asia and cultivated in the United States as an annual for its aromatic and attractive leaves (Simon & Quinn, 1988).

Fresh, dried, and dehydrated leaves are used as a condiment, garnish, and flavoring ingredient. An essential oil can be extracted from the leaves and seeds; it is used as a flavoring agent or fragrance in perfumes, soaps, and creams. The commercial essential oil of parsley is largely derived from the seed or the herb harvested at seed formation, prior to ripening (Petropoulos *et al.*, 2004). Zheng *et al.*, (1992) found that as a major volatile aroma constituent of parsley essential oil, myristicin may be an effective cancer chemopreventive agent. Gazzani, (1994) found that parsley showed weak antioxidant activity in groundnut oil under various heating conditions.

Components of fresh parsley leaf scavenge superoxide anion in vitro (Campanella *et al.*, 2003). Supplementation of diets with fresh parsley leaf can increase antioxidant capacity of rat plasma (Hempelet *et al.*, 1999) and decrease oxidative stress in humans (Nielsen *et al.*, 1999). Methanol extracts of parsley scavenge hydroxyl radical in addition to protecting against ascorbic acid-induced membrane oxidation (Fejes *et al.*, 2000).

Parsley is an excellent source of three vital nutrients that are also important for the prevention of many diseases: carotenoids, tocopherol and ascorbic acid (Davey *et al.*, 1996).

Ascorbic acid, commonly known as vitamin C plays significant functions in the human body, though its function at the cellular level is not very clear. Ascorbic acid is needed for collagen synthesis, a production of certain hormones and of neurotransmitters, metabolism of some amino acids and vitamins, detoxification of toxic substances in the body, and proper function of the immune system (Holloway *et al.*, 1982). Epidemiological data is increasing on the effect of vitamin C in cancer (Simon *et al.*, 2001), blood pressure (Pietinen & Aro, 1990). This vitamin also helps the liver in the detoxification of toxic substances in the system, and the blood in fighting infections (Firuzi *et al.*, 2005). Ascorbic acid is important in the proper function of the immune system (Walingo, 2005). Vitamin C antioxidant plays an important role in controlling the oxidative stress (Panayiotidis and Collins, 1997). It can also protect DNA against damages induced by various chemicals (Blasiak and Kowalik, 1999) and it can scavenge a variety of free radicals and oxidative molecules such as hydroxyl radicals ($\cdot\text{OH}$), superoxide anions (O_2^-) sulphhydryl radicals, oxidized LDL, and others (Frei, 1999). People are tempted to over-consume vitamin C because of its health benefits. Miniscule increases in blood vitamin C levels decrease the risk of death from all conditions [Gorton & Jarvis, 1999].

This study aimed to evaluate the changes associated with the early response to ovalbumin challenge and to investigate the anti-asthmatic effects of parsley, on the oxidant/antioxidant profile in an induced model of asthma.

MATERIALS AND METHODS

Wistar albino male 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 a 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 was 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 20 mg/Kg body weight of Parsley extract daily for two weeks according to Heinerman, 1996 & Day, 2005. The other group was administered 180mg/Kg body weight of ascorbic acid daily for two weeks according to Abdelmoneium *et al.*, 1997.

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) [37], carbonyl protein according to Levine *et al.*, 1990 [38], malondialdehyde according to Vento *et al* 2000 [39], 8 isoprostaglandin according to Lawson & Maxey 1996 [40], hydrogen peroxide according to Mc Namara, & Augusteyn, (1984) [41] and nitric oxide according to Miles *et al.*, 1996 and Maeda *et al.*, 2004 [42,43]. Whole blood samples were assayed for hemoglobin according to Van Kampen & Zijlstra (1961) [44], glutathione peroxidase according to Kraus & Ganther (1980) [45] and super oxide dismutase according to Suttle 1980 [46].

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 ± 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.

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). However, the decrease in the whole blood glutathione peroxidase activity did not reach a statistical significant level between the same comparative groups.

Wheezing episodes were diminished after the treatment with both Parsley and Vitamin C. 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).

On comparing the studied parameters in groups administered Parsley with those administered Vitamin C, there was a statistical significant difference in hemoglobin, carbonyl protein and superoxide dismutase clarified by statistical significant level P < 0.05 and P < 0.001 respectively. Consequently, nitric oxide, malondialdehyde, isoprostane, hydrogen peroxide and glutathione peroxidase did not show such difference.

Table 1: Investigated parameters in the studied groups.

	Control	Ova	Parsley	Vitamin C
Hemoglobin	15.25±0.17	10.9±0.22 ^{a**}	13.35±0.35 ^{a** ,b**}	12.16±0.29 ^{a** ,b* ,c*}
Nitric oxide	54.6±2.9	115.4±4.6 ^{a**}	67.5±5.8 ^{a** ,b**}	76.5±5 ^{a* ,b**}
Hydrogen peroxide	83.6±1.67	137.4±5.2 ^{a**}	91.01±5.98 ^{b**}	93.3±6.4 ^{b**}
Malondialdehyde	2.4±0.21	11.8±0.82 ^{a**}	6.5±0.35 ^{a**}	6.4±0.27 ^{a**}
Isoprostane	230.2±19.1	767.7±14.2 ^{a**}	442.7±41.2 ^{a** ,b**}	428.7±34 ^{a** ,b**}
Carbonyl group	0.51±0.019	0.96±0.07 ^{a**}	0.42±0.03 ^{b**}	0.53±0.03 ^{b** ,c*}
Glutathione peroxidase	137.8±8.1	106.9±4.5	182.6±8.5 ^{b*}	180.7±9.7 ^{b*}
Superoxide dismutase	345±11.5	195.8±9.3 ^{a**}	435.3±17.38 ^{a** ,b**}	311.7±8.7 ^{b** ,c**}

Data are represented as mean ± SE. a comparison vs control, b vs ova, c vs parsley.

*P < 0.05; ** P < 0.001

The correlation between the investigated oxidant and antioxidant status using Pearson’s correlation is represented in table (1). It reveals a highly statistically significant negative correlation between antioxidant parameters represented by superoxide dismutase and glutathione peroxidase 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. Scatter plot diagrams of these data are represented in figure (2).

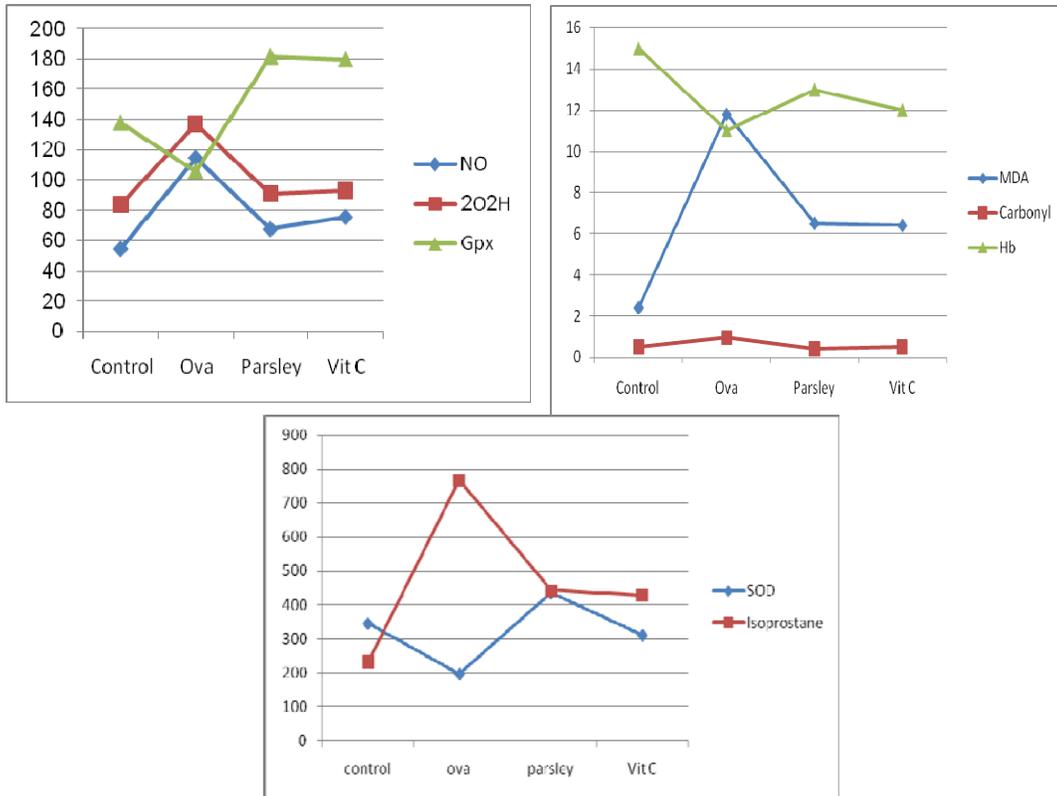


Fig. 1: Comparison of the studied parameters in all groups

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). In this study, we demonstrated the presence of early airway hyperresponsiveness or bronchoconstrictive responses induced by ovalbumin. Furthermore, our data clearly show that the increase in airway hyperresponsiveness caused by ovalbumin is improved upon treatment with parsley extract as previously reported by Malik *et al.*, 2008.

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

	Superoxide dismutase	Malodialdehyde	Isoprostane	Carbonyl group	H2O2
Hemoglobin	0.278*	-0.512**	-0.563**	-0.512**	-0.298*
Nitric oxide	-0.404**	0.547**	0.496**	0.488**	0.465**
Isoprostane				0.568**	0.444**
Malodialdehyde			0.677**	0.569**	0.595**
Glutathione peroxidase	0.181	-0.126	-0.009	-0.308*	-0.242
Superoxide dismutase		-0.530**	-0.344**	-0.657**	-0.488**

*P \square 0.05; ** P \square 0.001

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,

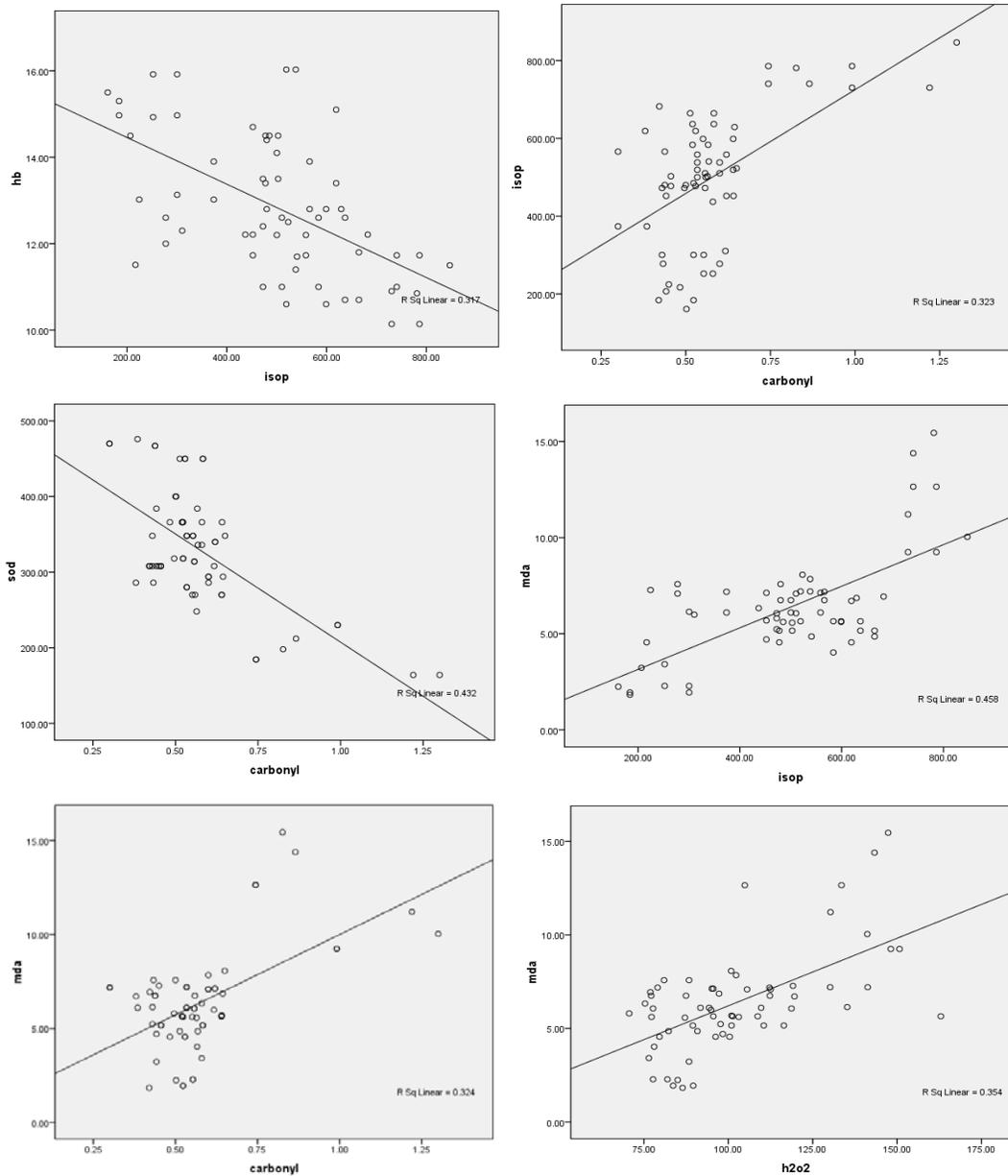


Fig. 2: Scatter plot of the correlation between investigated parameters in the studied groups.

eosinophilia, airway hyperreactivity, or epithelial cell metaplasia (Follettie *et al.*, 2006 and 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). 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 and Rahman, 2006). Although the antioxidant capacity of airway epithelial cells is excellent, upon repeated and continued 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. Increased dietary intake of ascorbic acid has been shown to improve lung function in asthma patients (Yadav *et al.*, 2009).

Fejes *et al.*, (1998) and Wong & Kitts (2006) investigated the in vitro antioxidant effect of various extracts prepared from different vegetative organs of parsley and observed that the essential oil plays a significant role in the scavenging effect.

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).

The present work has shown that hydrogen peroxide level was elevated in bronchial asthma induced group, and falls back by the administration of both parsley and vitamin C.

H₂O₂ can cause cellular damage and oxidize protein thiol groups, thereby altering cellular functions and activating intracellular signaling molecules. For example, in vitro experiments have revealed that exogenous H₂O₂ reverses interleukin (IL) 5-mediated survival and accelerates constitutive apoptosis of human eosinophils (Nagata, 2005). H₂O₂ can also stimulate eosinophil adhesion as an autocrine or paracrine mediator via the upregulation of β 2 integrin (Lee *et al.*, 2003). Many studies using various systems have also shown that exogenous H₂O₂ can activate intracellular signaling molecules associated with cellular death. For example, H₂O₂-mediated apoptosis in mouse fibroblasts or human neuroblastoma cells has been found to occur as a result of the activation of extracellular signal-regulated kinases (ERK) 1 and 2—mitogen-activated protein kinase (MAPK) (Ruffels *et al.*, 2004). H₂O-induced chondrocyte apoptosis also requires caspase activation (Lo and Kim, 2004).

Parsley significantly increased superoxidase dismutase (SOD) activity with significant decrease in malondialdehyde (MDA) content. Moreover, nitric oxide content was remarkably decreased as compared to the OVA group. Nader and his collaborators 2012, revealed a markedly decreased inflammatory cell accumulation in bronchoalveolar lavage (BAL) fluid and in the lungs, as revealed by histopathological examination. Furthermore, the levels of MDA, nitric oxide content as well as the mRNA expression of inducible nitric oxide synthase (iNOS) were remarkably decreased. While a significant increase in SOD activity was also proved.

No significant differences in erythrocyte glutathione peroxidase activity which evaluate reactive oxygen species removal antioxidant enzymes acting on the antioxidant defense system, were observed before and after supplementation. In a previous study by Fernández-Pachón *et al.*, 2009, which evaluated the effect of red wine supplementation in healthy adults for 7 days, the red blood cell catalase and SOD activation were shown to significantly increase, but there were no significant changes in the activation of GSH-Px. It was also reported that antioxidant enzymes activation in red blood cells was not improved in healthy adults after supplementation with fruit and vegetable (Freese *et al.*, 2008).

It is not easy to interpret the meaning of the increase or decrease of antioxidant enzymes in this nutrition intervention study. This is the case because the human body works to maintain the physiological homeostasis, and many antioxidant enzymes are involved and used up when the oxidizing stress is high, but in some cases it may increase the generation of antioxidant enzymes. Therefore, the activation of antioxidant enzymes may not be a sensitive biomarker of the antioxidant nutrition status in the human body and therefore other surrogate markers of oxidative stress must be used.

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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