Smokeless Tobacco Enhance Allergic Inflammation, Exacerbation of Asthma and Oxidative Stress in Asthmatic Patients from Algeria

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Abstract

Background and objective

Despite their effects on human health, the link between smokeless tobacco (ST) consumption and asthma severity in asthmatic patients is still unknown. Thus, the present study aims to complete the lack of information by investigating the aggravation of inflammation, exacerbation of asthma, oxidative stress and cytotoxicity induced by ST in asthmatic patients.

Methods

The study recruited 80 male volunteers residing in Annaba town, Algeria, divided into four groups by using a questionnaire, each group consists of 20 male volunteers. Herein, biochemical parameters, hematological parameters, C-reactive protein (CRP), total IgE, interleukin-5 (IL-5), nitric oxide (NO) and oxidative stress were measured.

Results

The obtained results showed that ST clearly enhanced lung inflammation and exacerbation of asthma through total IgE, IL-5 and CRP increased production. In addition, ST was found to intensify oxidative stress via increased lipid peroxidation and decreased reduced glutathione (GSH) levels. Likewise, the biochemical and hematological parameters results showed that ST causes damage and inflammation to tissues.

Conclusion

Therefore, our study reveals that ST obviously enhances allergic inflammation in patients suffering from asthma.

Introduction

Asthma is a heterogeneous disease diagnosed by the presence of intermittent symptoms of wheeze, cough and chest tightness [1, 2] Typically related to reversible airflow obstruction, usually resolves spontaneously or with asthma treatment [3, 4]. Prevalence of asthma is high (4.3%) in both children and adults and it affects about 300 million people of all ages worldwide. In 2025, the number of affected people would jump to more than 400 million [5]. It is increasing by 50% per decade and is considered a very serious public health problem [6]. World Health Organization (WHO) estimates that 255,000 deaths occur per year due to asthma [7]. However, ratio of death due to asthma is higher (i.e., 80%) in low or middle-income countries [7]. In Algeria one and a half million people with asthma with 1000 deaths per

year. In addition, asthma in Algeria ranks third among chronic diseases after high blood pressure and diabetes, with a prevalence of 9% and a morbidity rate of 3.75% [8].

Allergic inflammation is also frequently associated with an increased generation of reactive oxygen species (ROS) and the biochemical environment in the asthmatic airways is favorable for free radical mediated reactions [9]. It has been shown that inflammation caused by increased oxidative stress occurs in the airways of patients with asthma [10].

Smoking and smokeless tobacco (ST) products are the two modes of tobacco consumption that have been assessed throughout the globe and the practice of ST is common [11, 12]. In total, there are 1.4 billion tobacco users aged 15 years and above worldwide, 1.07 billion smokers and 367 million smokeless tobacco users. A small number of them use both smoked and ST [13]. In Algeria, the smokeless tobacco traditionally consumed is chewing tobacco. The current prevalence of smokeless tobacco use is 10.4%. It is 21% for men and 0.4% for women. This consumption is 9.4% among 24–34 years old, then rises to 13.6% at the age of 45–54 years to reach 10.2% at the age of 55–64 years. This consumption is higher in rural areas (11.6%) compared to urban areas (8.5%) [14].

The main ingredients of smokeless tobacco products are nicotine, alkaloid, tobacco-specific N-nitrosamines (TSNA), N-nitrosamino acids, volatile N-nitrosamines, formaldehyde, acetaldehyde, hydrocarbons, and heavy metals like polonium-210 are described [15]. Exactly how ST mediates lung damage is not well defined. However, presumably the direct contact of the respiratory epithelium with ST toxic compounds, even when it is not burned or volatilized, can cause inflammatory changes [16].

It has been well proven that the short-term administration of ST in a rat model of allergic asthma causes the exacerbation of asthma as demonstrated by interleukin-4 (IL-4) and Nitric oxide (NO) increased production. Actually, Khaldi et al. (2018) showed that ST was found to intensify the oxidative stress state induced by Ova-challenge in rats, which was proven not only by augmenting lipid peroxidation and protein oxidation, but also by altering the non-enzymatic and enzymatic antioxidant status. Furthermore, the aggravation of inflammation and oxidative stress was obviously demonstrated by the histopathological changes observed in the lungs [10]. To the best of our knowledge, no study has been conducted to show the correlations between ST consumption and asthma exacerbation in asthmatic patients. Thus, the aims of this present study are to explore the immunological, biochemical, biophysical and histopathological alterations due to the chronic consumption of smokeless tobacco in asthmatic patients.

Materials And Methods

Study subjects and study description:

The study recruited 80 male volunteers divided into four groups by using a questionnaire. Each group consists of 20 male volunteers: group I- normal healthy control, group II- asthmatic patients, group III- smokeless tobacco users, group IV- asthmatic patients and smokeless tobacco users. The experimental

subjects are performed on adult males between the ages 25 - 50 years residing in Annaba town, Algeria. All the subjects were included smokeless tobacco users for at least five years. The commonly available smokeless tobacco products are chewing 5-20 times per day at 3-10 g each time. In this study, healthy controls are subjects who did not abuse tobacco or alcohol and had not been exposed to any kind of chemicals. Moreover, they had the same age, sex, and socioeconomic status. All protocols in this study were used in accordance with the guidelines of the Committee on Use of Laboratory Animals and approved under the CNEPRU project (D01N01UN230120150006) by the Ethical Committee of DGRSDT at the Algerian Ministry of Higher Education and Scientific Research.

Collection of blood samples and estimation of biochemical parameters

After obtaining proper written informed consent, a volume of 5 ml venous blood sampling was collected from the subjects. Then, blood samples were centrifuged for 15 min at 3000 RPM and stored at −20◦ C. The following biochemical parameters in plasma were estimated based on established spectrophotometric and automated procedures, approved by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). Aspartate amino transferase (ASAT) and alanine amino transferase (ALAT) **[17, 18]**, total proteins **[19]**, alkaline phosphatase (ALP) **[20]**, urea **[21]** and creatinine **[22]**, were estimated using standard kits from Spinreact (SPINREACT. S.A/SAU. Ctra. Santa Coloma, 7 E-17176SANT ESTEVE DE BAS (GI) SPAIN). All biochemical tests were performed in plasma samples using ARCHITECT ci8200 Abbott Diagnostics Autoanalyzer.

Hematological parameters

Blood samples in EDTA tubes analyzed for hematological parameters were determined by an electronic hemocytometer (Full Automatic Blood Cell Counter MODEL ERMA INC, PCE-210N).

C-Reactive protein levels

C-reactive protein (CRP) levels were measured on a Hitachi 911 analyser using a latex slide agglutination test kit from Spinreact Spain (CRP-Latex, Ref. ID: 1200305).

Total IgE levels

Plasma IgE levels was measured using ELISA (Enzyme linked immunosorbent assay) commercially purchased kit (Roche diagnostics, Meylan, France) and the test was performed according to the manufactured instructions. Briefly, 10 µl of plasma samples were added to the Ag-coated wells, the plates were incubated, and bound IgE were detected with biotinylated anti-human IgE. Streptavidin-peroxidase conjugates were added, the bound enzymes were detected by addition of tetramethylbenzidine substrate system and absorbances were read at 450 nm. Absorbances were converted to arbitrary units.

Interleukin-5 (IL-5) measurement

Plasma IL-5 levels were measured, according to the manufacturer's protocol, using Novex Rat IL-5 ELISA (enzyme-linked immunosorbent assay) commercial kit purchased from Invitrogen (Camarillo, CA, USA). After measuring the optical density at 450 nm, the concentrations of IL-5 were determined by interpolation from a standard curve, with all data expressed in pg/mL.

Nitric oxide (NO) measurements

The NO production in plasma was determined by the detection of nitrite ($NO2^{-}$) concentration from the Griess reaction **[23]**. The absorbance was measured at 530 nm from an automatic microplate reader (Mindray MR-96A). Nitrite concentration was compared to a sodium nitrate standard curve.

Estimation of lipid peroxidation levels

The lipid peroxidation (LPO) was evaluated using the MalonDiAldehyde (MDA) level as a final product in the plasma. MDA reacts with thiobarbituric acid (TBA) as a reactive substance (TBARS) to produce a red-colored complex **[24]**. The absorbance was read at 530 nm.

Reduced glutathione (GSH) levels

Reduced glutathione (GSH) contents of plasma were estimated by the colorimetric method of Jollow and al. (1974) **[25]** based on the development of a yellow color when DTNB [5,5-dithiobis(2nitrobenzoic acid)] was added to compounds containing sulfhydryl groups. The absorbance was recorded at 412 nm.

Results

Hematological estimations

Data presented in (Table 1) indicates that ST users showed a significantly increased number of leucocytes, monocytes and granulocytes, as well as hemoglobin and IDR levels compared to the control group. However, we noticed a decreased number of RBC, HCT and VMP levels yet compared to the control. Moreover, the data shows a significant increase in the number of WBC, granulocytes and a significant decrease in HGB levels in asthmatic smokeless tobacco users compared to the asthmatic group.

Biochemical parameters

The obtained results (Table 2) showed a significant increase in ASAT, ALAT and GGT activity in the ST users group compared to control. Moreover, the asthmatic group showed a significant increase of ASAT, ALAT, ALP, GGT activity and a considerable decrease in urea levels compared to the control. Nevertheless, we noticed a significant increase of urea levels in asthmatic ST users compared to the asthmatic group.

CRP levels

The obtained results showed a significantly increased CRP levels in ST users compared to control. Moreover, in asthmatic ST users, CRP levels were significantly increased compared to the asthmatic group (Fig. 1).

Total IgE levels

In our study, total IgE showed a highly significant increase in ST users, asthmatic and asthmatic ST users groups compared to control. Furthermore, total IgE was significantly increased in asthmatic ST users compared to the asthmatic group (Fig. 2).

IL-5 levels

The obtained results indicate a significant increase of IL-5 levels in asthmatic and asthmatic ST users groups compared to control. In addition, IL-5 levels were significantly increased in asthmatic ST users compared to the asthmatic group (Fig. 3).

NO levels

Our results showed that NO levels were significantly increased in ST users, asthmatic and asthmatic ST users groups compared to control (Fig. 4).

MDA levels

Our results showed a significant increase of MDA levels in ST users, asthmatic and asthmatic ST users compared to control. Furthermore, MDA levels were significantly increased in asthmatic ST users compared to the asthmatic group (Fig. 5).

GSH levels

In the present study, GSH levels were significantly decreased in ST users, asthmatic and asthmatic ST users groups compared to control. Moreover, GSH levels showed a significant decrease in asthmatic ST users compared to the asthmatic group (Fig. 6).

Discussion

Despite there are several clinical associations, the direct or indirect toxicity of the lung by ST is certainly not at the level caused by smoked tobacco. Inflammatory changes leads to lung cancer, cardiovascular, non-cancer respiratory diseases, chronic obstructive pulmonary disease (COPD) and digestive system-related diseases [16]. It is known that cigarette smoke causes the exacerbation of asthma as determined by functional airway hyperreactivity and increased levels of blood eosinophilia in Ova-sensitized mice [26]. However, studies of the relationship between ST and asthma are very rare in the literature. In fact, in our previous study, we showed that shot-term administration of ST in a rat model of allergic asthma clearly contributed to the worsening of lung inflammation, intensifies the oxidative stress state induced by Ova-challenge in rats, which was obviously affirmed by the lung histopathological changes observed

in this study [10]. To the best of our knowledge, there is no study available showing ST consumption effects in asthmatic patients.

The findings of Ukoha et al. (2012) [27] indicated that chronic tobacco consumption might put the body at some risk of adverse hematological and homeostatic conditions. Mukherjee reported that the adverse effects of ST on hematological parameters are not less than smoking [28].

Nicotine as an element present in all tobacco products might alter the suprarenal glands to produce more catecholamine [29], which may influence leukocytosis causing damage and inflammation to tissues [30].

In the present study, WBC count was significantly increased in ST users as compared to controls. A similar significant increase in WBC count was also seen in previous studies [12, 31–34].

The granulocytes, mainly neutrophils, significantly increased in the present study in ST users compared to control, which may be linked with continuous inflammation of tissues. Neutrophils are well known to produce cytotoxic material which harmful to lung functions [31].

In our study, monocytes number were significantly increased in ST users than controls. Few studies confirm the association of ST with higher levels of monocytes [35, 36], which indicates the presence of infection in the individuals who consume ST.

However, lymphocytes did not show any significant change in ST uses in the present study. According to previous studies, inadequate pulmonary function in ST users might be responsible for stimulating erythropoiesis for fulfilling the demands of oxygen to the tissues [30].

The increased hemoglobin (Hb) levels in ST users in contrast to the control group in the present study is possibly secondary to hypoxic stimuli exerted by ST. In fact, high levels of carbon Monoxide (CO) are present in smokeless tobacco as well as in cigarette smoke. Furthermore, smokers may possess continuously rise levels of carboxy – Hb in the blood [37]. CO lowers the affinity of hemoglobin for oxygen by binding with Hb to form carboxy – Hb. This affects the capacity of Hb to carry and deliver oxygen to different tissues of the body [38]. Changes in arterial oxygen tension are known to affect erythropoietin production. The production of erythropoietin is known to be affected by changes in arterial oxygen tension.

In hypoxic conditions erythropoietin production is enhanced as a result more erythrocytes are produced by erythropoiesis [28]. Thus, the increase in Hb levels is quite obvious and expected.

In the present study, hematocrit (HCT) levels were significantly decreased as compared to control. ST is also well known for its effects on physiological processes. In a recent study, ST users revealed an alteration in RBC morphology. Electron microscopy scanning demonstrated modifications in the RBC membranes with fine “bubble-like” protrusions lacking their discoid shape. ST ingredients disrupt individuals’ cellular metabolism that contributes to shape changes and scale which have enormous consequences in terms of health maintenance [35].

This RBC morphology alteration can explain the significantly increased RDW levels in ST users as compared to the control in the present study.

Mean platelet volume (MPV) is an essential indicator of platelet activation. The size of the platelet, activity and the function of the platelet are correlated. Larger platelets are more active than smaller ones [39]. There are very few studies relating to the effect of ST on platelets. In the present study, MPV levels are significantly decreased in ST users and the asthmatic group as compared to the control. This is in agreement with a previous study [40].

C-reactive protein (CRP) is an acute-phase reactant secreted by hepatocytes in response to circulating inflammatory cytokines. It has long been used clinically to evaluate the presence and degree of inflammation because CRP blood levels increase as much as 1.000-fold within 24 hours after the onset of inflammation [41].

In our present study, CRP levels are significantly increased in asthmatic patients as compared to the control group. Our results are consistent with previous studies which confirmed that CRP is increased in asthmatic patients than in healthy control, and may be a useful biomarker of airway inflammation in non-smoking asthmatic patients without complications, such as heart disease, hypertension, hyperlipidemia, chronic obstructive pulmonary disease, or infection [42, 43].

Smokeless tobacco causes a significant rise in CRP levels and inflammatory cells. The significant rise of CRP among ST users can be attributed to the inflammatory response in the body [35]. Thus, our results showed that CRP levels are significantly increased in ST users and asthmatic ST users groups as compared to the control group. Our findings are similar to those of Costello et al. (2013) and Furie et al. (2000), in which authors reported that both smokeless and addictive tobacco use leads to higher levels of CRP [44, 45].

IgE is an immunoglobulin that plays a significant role in chronic inflammatory allergic diseases and acute allergic reactions [46]. It is a central mediator in atopic asthma, which is produced by sensitized allergen-specific B cells [47]. Our study showed a highly significant increase of total IgE levels in patients with allergic asthma in comparison to the control. These findings are in accordance with previous studies [48–50]. This is due to the stimulated Th2 cells that are known to produce higher levels of s IL-4 and IL-13 which mediate the development of eosinophils and stimulate B-cells to secrete the specific immunoglobulin E [48]. The eosinophilic phenotype is associated with an intense production of IL-5 and IL-13 [4].

In addition, our results showed that total IgE levels are significantly increased in ST users and asthmatic ST users groups in comparison to the control group. Our results are in agreement with previous studies [51, 52]. Furthermore, our data showed a significant increase of total IgE in the asthmatic ST users group as compared to the asthmatic group. This elevation among ST users can be explained as nicotine the main component in tobacco products increases mucosal permeability allowing easier and greater access of allergens to sub epithelial lymphoid tissue and this rise in IgE levels indicates an increased probability

of type 1 hypersensitivity reaction (allergy), which also explains the allergy symptoms experienced ST users [52].

IL-5 is the most important Th2 cytokine associated with eosinophils, and it can regulate most aspects of eosinophil behavior including eosinophil growth, maturation, differentiation, survival, and activation [47]. Therefore, this cytokine exerts key functions in the pathogenesis of eosinophilic asthma, which is often therapeutically responsive to corticosteroids because of its effective ability to induce eosinophil apoptosis [53]. Interleukin-5 acts as a homodimer, and is essential for the maturation of eosinophils in the bone marrow and their release into the blood [54].

In the present study, IL-5 levels were significantly increased in asthmatic patients in comparison to the control. Our results are in agreement with previous studies, which demonstrated that higher serum IL-5 concentrations were detected in subjects with severe disease in comparison to healthy control subjects [54, 55].

Allergen inhalation increases the production of IL-5 in the airways as measured in bronchoalveolar lavage cells [56] and induced sputum [57]. Interestingly, eosinophils are one of the cell types responsible for this rise. Moreover, allergen inhalation rises the number of peripheral blood eosinophils and lymphocytes containing intracellular IL-5 [58]. The relatively high numbers of lymphocytes positive for IL-5 suggest that IL-5 is contained not only in $CD4^+$ T_H2 cells but also in other lymphocytes, including $CD8^+$ and $CD4^-$ $CD8^-$ cells [59]. In addition, it has been shown that IL-5 is the predominant eosinophil active cytokine present in BAL fluids during allergen-induced late-phase inflammation and may play a key role in the pathophysiology of allergen-induced, eosinophil-predominant airway inflammation [60].

Our results also showed a significant increase of IL-5 levels in asthmatic ST users in comparison with the control group and also with the asthmatic patients. Previous studies have demonstrated that tobacco smoking is associated with the increase of the bronchoalveolar levels of IL-5 in: acute eosinophilic pneumonia [61], in a model of house dust mite asthma [62], in allergic rhinitis mice [63]. Furthermore, Cozen et al., showed that when genotype, age, and gender are accounted for, smoking appears to be associated with increased capacity to secrete IL-5 [64]. Their study supports earlier observations [65, 66] and suggests that tobacco smoke may exacerbate or even lead to asthma through “priming” of immune cells toward a Th2 phenotype, possibly through an IgE independent pathway.

Nitric oxide (NO) is now well recognized for its involvement in diverse biological processes, including vasodilation, bronchodilation, and regulation of inflammatory-immune processes [67]. Thus, it is not surprising that the role of NO in asthma has been under investigation. Accumulating evidence indicates that NO plays a role in the regulation of airway function both in health and disease. Indeed, exhaled NO has been detected in normal subjects and asthmatics [68–70].

In the present study, NO levels were significantly increased in asthmatic patient compared to the control group. These findings are in concordance with many previous studies which have shown increased NO levels in asthmatic patients [71–73]. The exact pathophysiological role of NO in the airways and lungs is

complex. On the one hand, it may act as a proinflammatory mediator predisposing to the development of airway hyperresponsiveness (AHR) [74, 75]. On the other, under physiological conditions NO acts as a weak mediator of smooth muscle relaxation, and protects against AHR [76]. In exhaled air, NO appears to originate in the airway epithelium, as a result of NOS_2 up-regulation which occurs with inflammation [73, 77]. Thus, exhaled NO may be regarded as an indirect marker for up-regulation of airway inflammation [71].

Furthermore, our results showed a significant increase in NO levels in ST users compared to the control group. As well as, in asthmatic ST users compared to asthmatic patients. Many previous studies have demonstrated that ST consumption led to elevated NO levels compared to control [78–80].

Smokeless tobacco includes specific chemicals like polycyclic aromatic hydrocarbons, N-Nitrosamines aromatic amines, ethylene oxide, 1,3-butadine, and other tobacco-specific nitrosamines. Tobacco causes increased generation of free radicals and reactive oxygen species, such as NO, superoxide anions, hydroxyl radicals, etc. [81]. In contrast, the major constituent of tobacco, namely nicotine and its active metabolite cotinine, have been shown to stimulate NO production neurologically and also shown to stimulate angiogenesis and promotes tumor growth thought to be mediated by the production of NO and other factors [82–84].

According to Chan et al. (2009) and Aldakheel et al. (2016) in patients suffering from asthma, the imbalance between reactive oxygen species (ROS) and antioxidants leads to in oxidative stress due to the development of airway inflammation secondary to the actions of inflammatory mediators [85, 86]. Oxidative stress is believed to play a crucial role in the pathophysiology of asthma because several of the characteristic changes in the airways can be produced by the actions of ROS [87].

Our results showed a significant increase in malondialdehyde (MDA) levels in asthmatic patients compared to the control. MDA has been widely studied as a major reactive aldehyde resulting from the peroxidation of biological membrane polyunsaturated fatty acids, It is used as an indicator to identify damaged tissues by a series of chain reactions [88]. Analyses confirmed that in patients with asthma, the MDA concentrations is high in several biological fluids [89–92]. These results indicate that increased production of the ROS may lead to increasing oxidative injury, which has been implicated in the pathogenesis of asthma [93].

Moreover, MDA levels in the present study showed a significant increase in ST users compared to the control as well as in asthmatic ST users compared to asthmatic patients. Several earlier studies have demonstrated that ST use increases lipid peroxidation in comparison with non-users [11, 94–96]. Furthermore, in our recent study, we have shown that ST administration to Ova-sensitized rats causes a significant increase of MDA levels compared to Ova-sensitized rats. In fact, nicotine and tobacco-specific nitrosamines could reinforce the increased ROS production, and decreased antioxidant defense leads to lipid peroxidation and protein oxidation. Likewise, several studies can be found that the various

ingredients of smokeless tobacco extract were more toxic than pure nicotine alone in the induction of ROS formation and disparity of redox state [97].

In contrast, the lung and blood are endowed with several antioxidants, including GSH, superoxide dismutase (SOD), catalase, vitamin E, and vitamin C, to opposite the oxidant-mediated response [98, 99]. Therefore, the increase of the oxidant burden rate associated with such inflammatory disease (asthma) may lead to physiological changes in serum levels of the antioxidant [93]. Glutathione (GSH) is considered a key molecule in the antioxidant pathways. This tripeptide is found in the cytosol and extracellular spaces, such as the lining fluid of the lung and plasma [100, 101].

The obtained results in the present study show a significant decrease in GSH levels in asthmatic patients compared to normal subjects. These data are in agreement with previous studies that reported that the levels of GSH in asthmatic patients decrease compared to the control subjects [93, 102–105]. This significant decrease of GSH levels in asthmatic patients may correlate with the asthma exacerbation and the low antioxidant defense. Moreover, the decrease in GSH levels in asthmatic patients may be due to increased consumption of GSH [106] or a lack of the amino acids found in the structure of glutathione [107]. Sackesen et al. (2008) observed lower levels in glycine and glutamic acid in children with asthma, and they suggested that this might result from excessive use of these amino acids for increased glutathione production to cope with free radicals [107]. Likewise, several studies have shown that oxidized glutathione (GSSG) and total glutathione (GSH and GSSG) levels are higher in the erythrocyte hemolysate, plasma, bronchial washing and BAL fluid of asthmatic patients [108–110]. Since it is already understood that increased levels of GSSG correspond to increased glutathione oxidation [102], and GSH level reduction, only GSH was considered in this study.

Furthermore, GSH levels in the present study show a significant decrease in ST users compared to the control group. These findings are in agreement with many previous studies which demonstrate that ST consumption reduces GSH levels in human and in rats [10, 111–113]. Therefore, the toxicity of ST in numerous organs in particular the lung might be linked to the formation of the radical species. The diverse elements of ST such as: tobacco, betel quid, areca nut, and catechu, among others, have also been reported to be toxic in experimental animals [114–116]. Moreover, during the metabolism of smoked tobacco many electrophiles are generated which are detoxified by the use of GSH [117]. Consequently, the decreased GSH levels increase the free radical burden due to ineffective removal of ROS from the tissues, which results in increased lipid peroxidation. In addition, enhanced lipid peroxidation with a concomitant decrease in reduced GSH is indicative of oxidative stress, which provides evidence to show the relationship between lipid peroxidation, tissue damage, and inflammation [118].

ALT (alanine transaminase) and AST (aspartate transaminase) are the liver enzymes, which play an important role in protein metabolism and are markers of liver function [119, 120]. Compared to the control group, the level of ALT and AST in asthmatic patients is elevated. This finding agrees with many previous studies [96]. The main causes of high ALT and AST values have been identified as hepatocellular damage and inflammation. However, levels of both enzymes are sometimes falsely lowered [121]. Similarly,

increased levels of LAP and GGT showed in asthmatic patients in the present study is an indicator of liver injury. Furthermore, the obtained results of the present study showed a significant increase of ALT, AST and GGT levels in ST users compared to the control group. Many previous studies showed elevated levels of these hepatic enzymes due to ST consumption [10, 96]. Elevated levels of ALT, AST and GGT in ST users might be related to damage and destruction of the liver tissue as ST contains ingredients of hepatotoxic agent, which induces microsomal enzyme of liver cells [122].

Uric acid the final product of purine degradation, acts as an antioxidant by virtue of its ability to tightly bound iron and copper [123]. Thus, the decreased levels of urea in asthmatic patients could be explained by its conjugation with ROS, the temporal order of antioxidant consumption in human blood plasma exposed to a constant flux of aqueous peroxy radicals is vitamin C, bilirubin, uric acid and vitamin E [124].

Conclusion

Overall, the findings of the present study demonstrated that ST clearly contributed to the worsening of lung inflammation through total IgE, interleukin-5 (IL-5) and C-Reactive protein (CRP) increased production. In addition, ST was found to intensify oxidative stress via increased lipid peroxidation and decreased reduced glutathione (GSH) levels. Likewise, the biochemical and hematological parameters results showed that ST causes damage and inflammation to tissues. Taken together, our results reveal that smokeless tobacco is a risk factor for exacerbation and aggravation of asthma in human patients. Finally, to the best of our knowledge, this is the first study exploring ST consumption effects in asthmatic patients. Thus, further studies are needed to better understand the relationship between ST and asthma.

Declarations

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Conflict of interest

The authors declare no competing interests.

Availability of data and material

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Authors' contributions of each author

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Taha Khaldi, Mahfoud Messarah and Amel Boumendjel. The first draft of the manuscript was written by Taha Khaldi and Amira Aicha Beya, all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval

All protocols in this study were used in accordance with the guidelines of the Committee on Use of Laboratory Animals and approved under the PNR project (33/DFPR/ATRSS) by the Ethical Committee of Thematic Agency for Research in Health Sciences.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent to publish

The authors affirm that human research participants provided informed consent for publication of the results of this study.

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Tables

Table 1. Hematological parameters.

Parameters	Groups			
	C	ST	A	A/ST
WBC (x 10^3/µL)	06.60 ± 0.38	08.00 ± 0.53*	06.67 ± 0.43	08.08 ± 0.43**#
RBC (x 10^6/µL)	05.74 ± 0.23	05.81 ± 0.21	06.15 ± 0.87	05.90 ± 0.19
HGB (g/dL)	15.99 ± 0.42	18.12 ± 0.82*	16.92 ± 0.59	15.70 ± 0.37#
HCT (%)	49.10 ± 1.06	39.90 ± 1.92***	51.95 ± 1.79	50.10 ± 1.75
Lymphocytes (x 10^3/µL)	02.23 ± 0.10	02.51 ± 0.13	02.38 ± 0.14	02.68 ± 0.15*
Monocytes (x 10^3/µL)	00.42 ± 0.03	00.61 ± 0.08*	00.41 ± 0.03	00.46 ± 0.04
Granulocytes (x 10^3/µL)	03.94 ± 0.30	04.86 ± 0.39*	03.87 ± 0.31	04.92 ± 0.32*#
Platelets (x 10^3/µL)	242.00 ± 13.98	235.65 ± 11.70	225.60 ± 11.28	223.75 ± 12.56
RDW (fL)	12.90 ± 0.29	15.36 ± 0.77**	13.52 ± 0.20*	13.57 ± 0.19*
MPV (fL)	08.48 ± 0.13	07.85 ± 0.15**	07.97 ± 0.12**	08.12 ± 0.14*

Values are given as mean ± S.E.M for groups of 20 each. Significant difference: all groups compared to the control one (*p<0.05, **p<0.01, ***p<0.001), compared to asthmatic patients group (#p<0.05).

Table 2. Plasma biochemical parameters.

Parameters	Groups			
	C	ST	A	A/ST
Total Proteins (g/dL)	57.50 ± 1.65	58.36 ± 1.66	58.15 ± 2.65	61.95 ± 1.86*
ASAT (IU/L)	13.05 ± 1.25	17.35 ± 1.54*	15.90 ± 0.97*	17.25 ± 1.23*
ALAT (IU/L)	04.20 ± 0.52	12.20 ± 1.05***	05.95 ± 0.66*	06.25 ± 0.74*
ALP (IU/L)	124.00 ± 8.27	129.60 ± 6.16	142.95 ± 7.20*	131.10 ± 7.83
GGT (IU/L)	19.25 ± 1.32	27.35 ± 1.62***	24.10 ± 1.42**	22.70 ± 1.06*
Urea (g/mL)	00.33 ± 0.02	00.32 ± 0.01	00.26 ± 0.01*	00.32 ± 0.01
Creatinin (mg/L)	08.25 ± 0.40	08.75 ± 0.76	08.65 ± 0.55	07.60 ± 0.43

Values are given as mean ± S.E.M for groups of 20 each. Significant difference: all groups compared to the control one (*p<0.05, **p<0.01, ***p<0.001), compared to asthmatic patients group (#p<0.05).

Figures

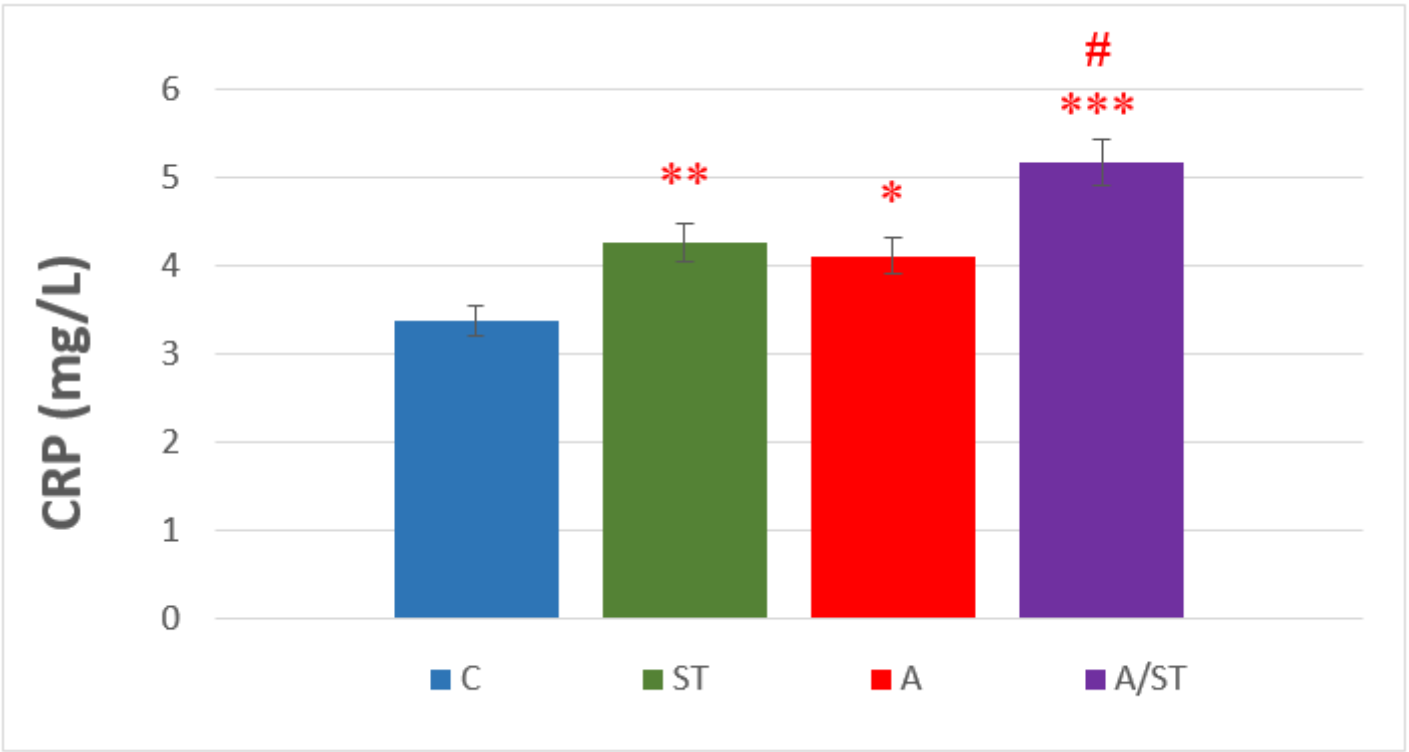

Figure 1

C-reactive protein levels (mg/L).

Significant difference: all groups compared to the control one (*p<0.05, **p<0.01, ***p<0.001), compared to asthmatic patients group (#p<0.05).

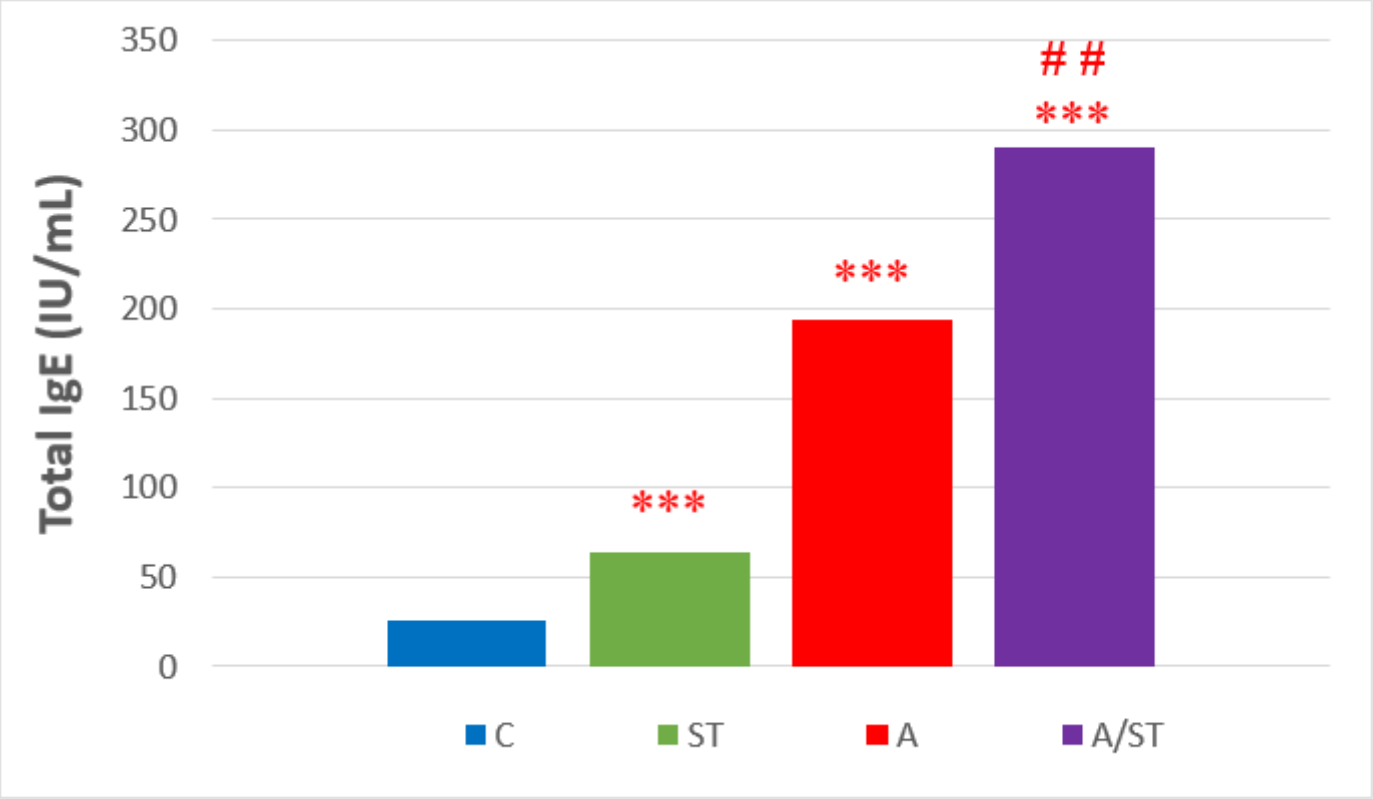

Figure 2

Total IgE levels (IU/mL).

Significant difference: all groups compared to the control one (***p<0.001), compared to asthmatic patients group (# #p<0.05).

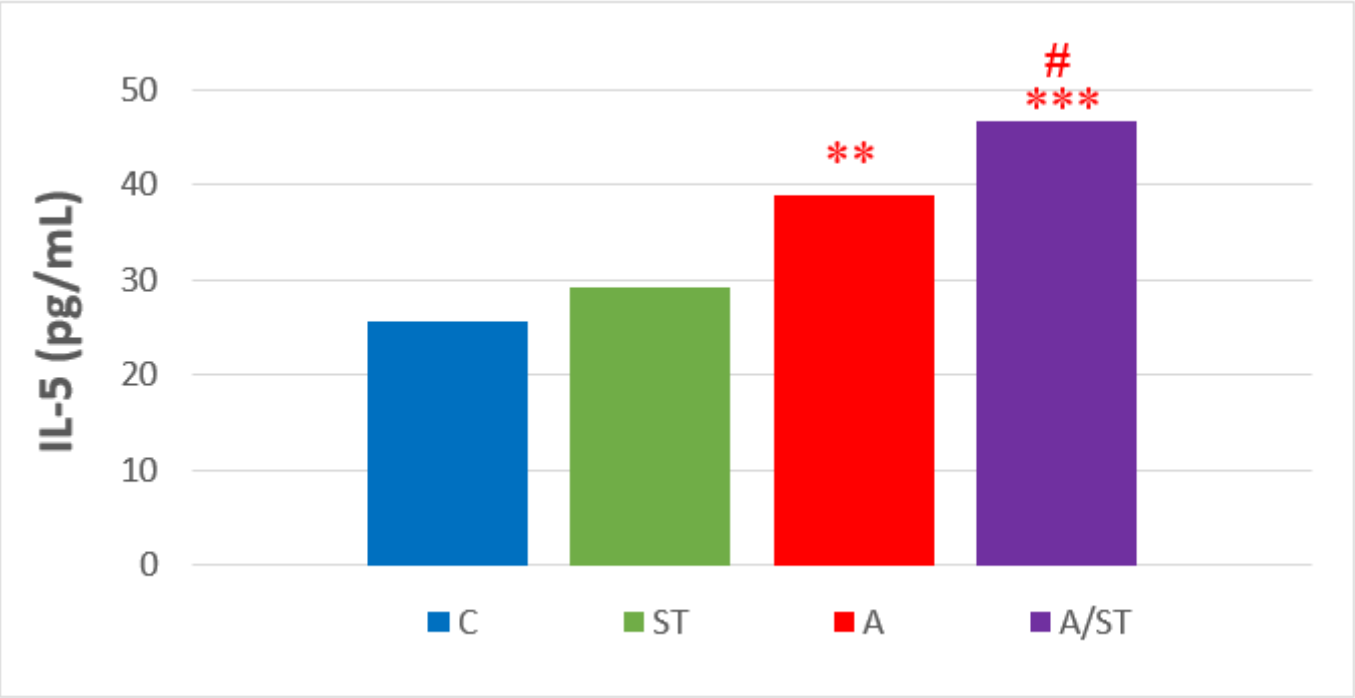

Figure 3

IL-5 levels (pg/mL).

Significant difference: all groups compared to the control one (**p<0.01, ***p<0.001), compared to asthmatic patients group (#p<0.05).

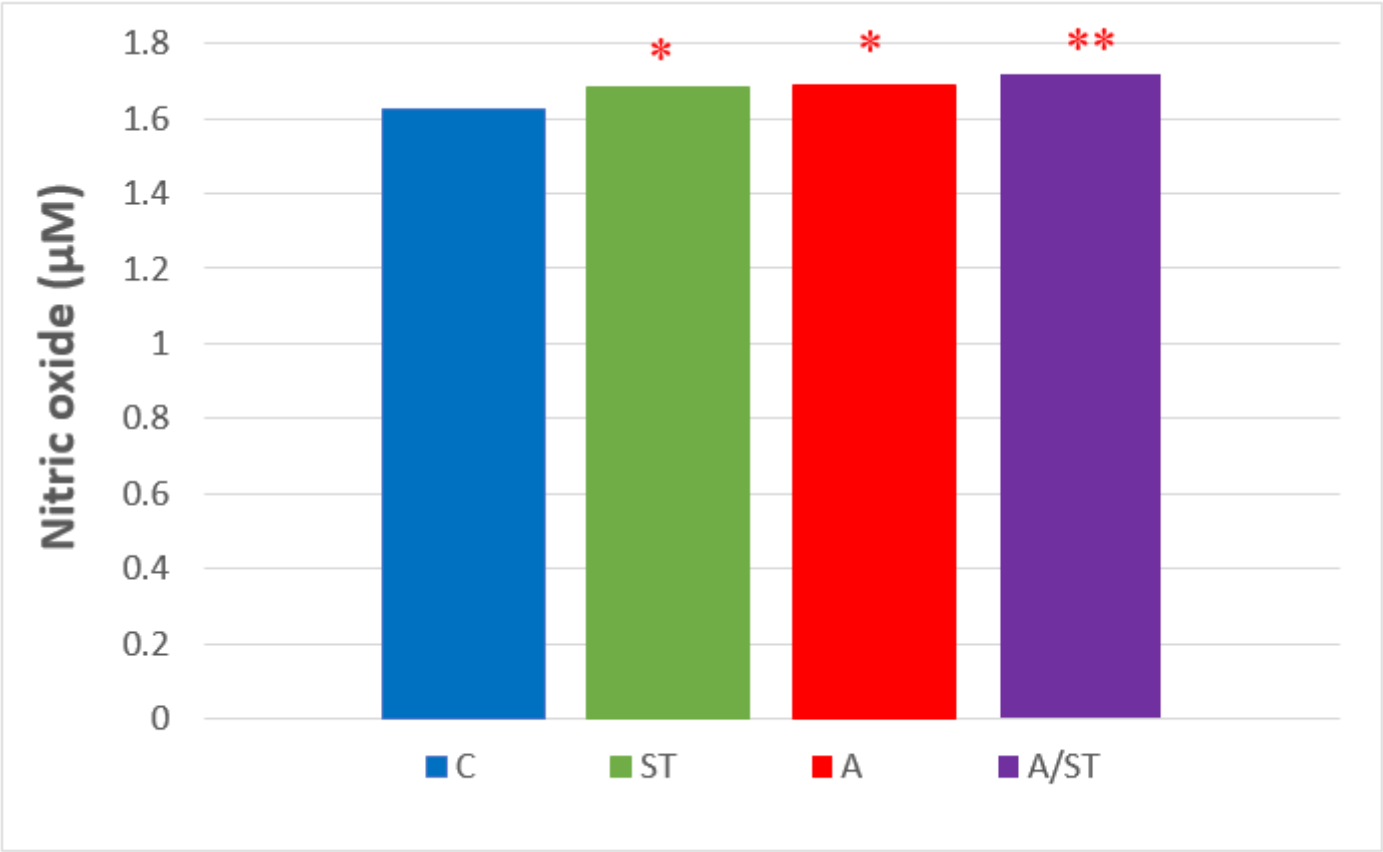

Figure 4

Nitric oxide levels (pg/mL).

Significant difference: all groups compared to the control one (*p<0.05, **p<0.01).

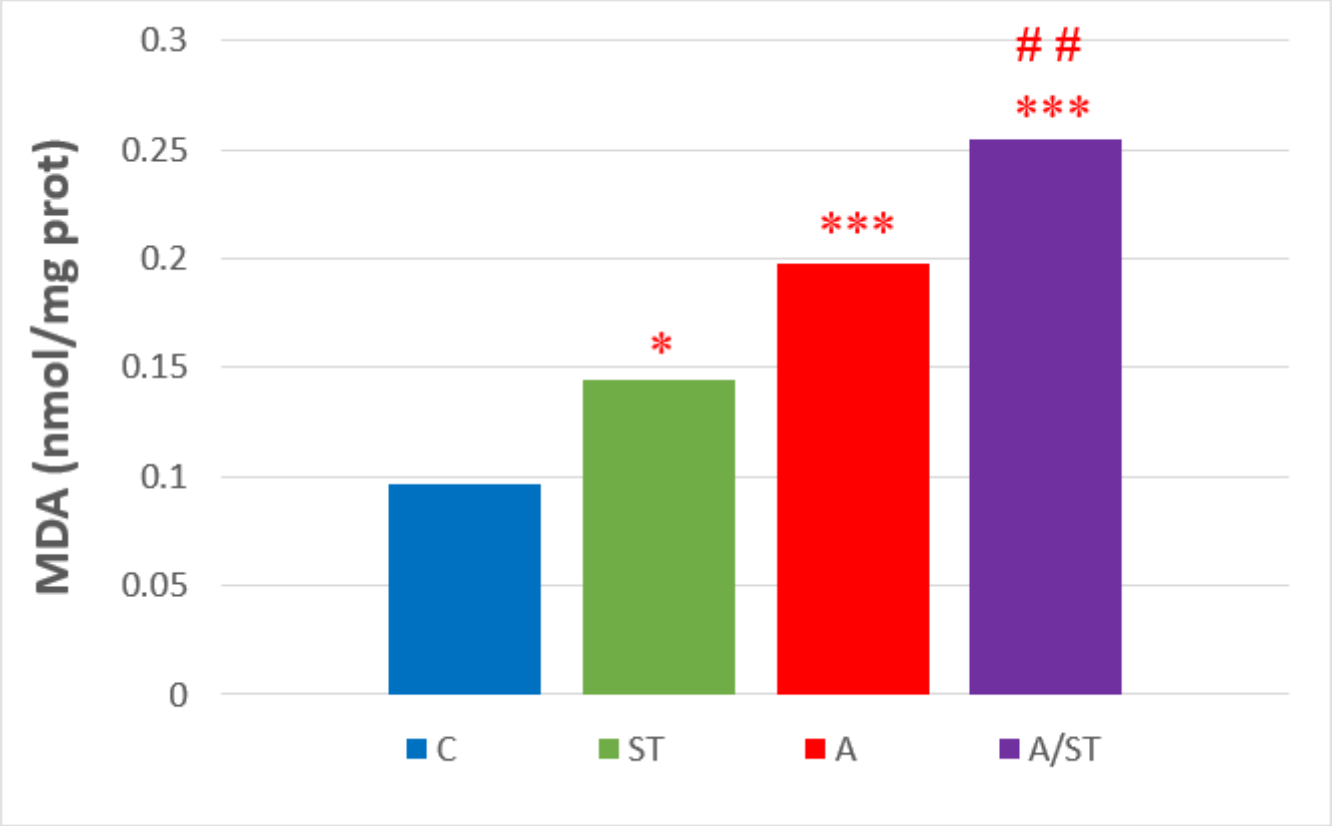

Figure 5

MDA levels (nmol/mg of prot).

Significant difference: all groups compared to the control one (*p<0.05, ***p<0.001), compared to asthmatic patients group (##p<0.01).

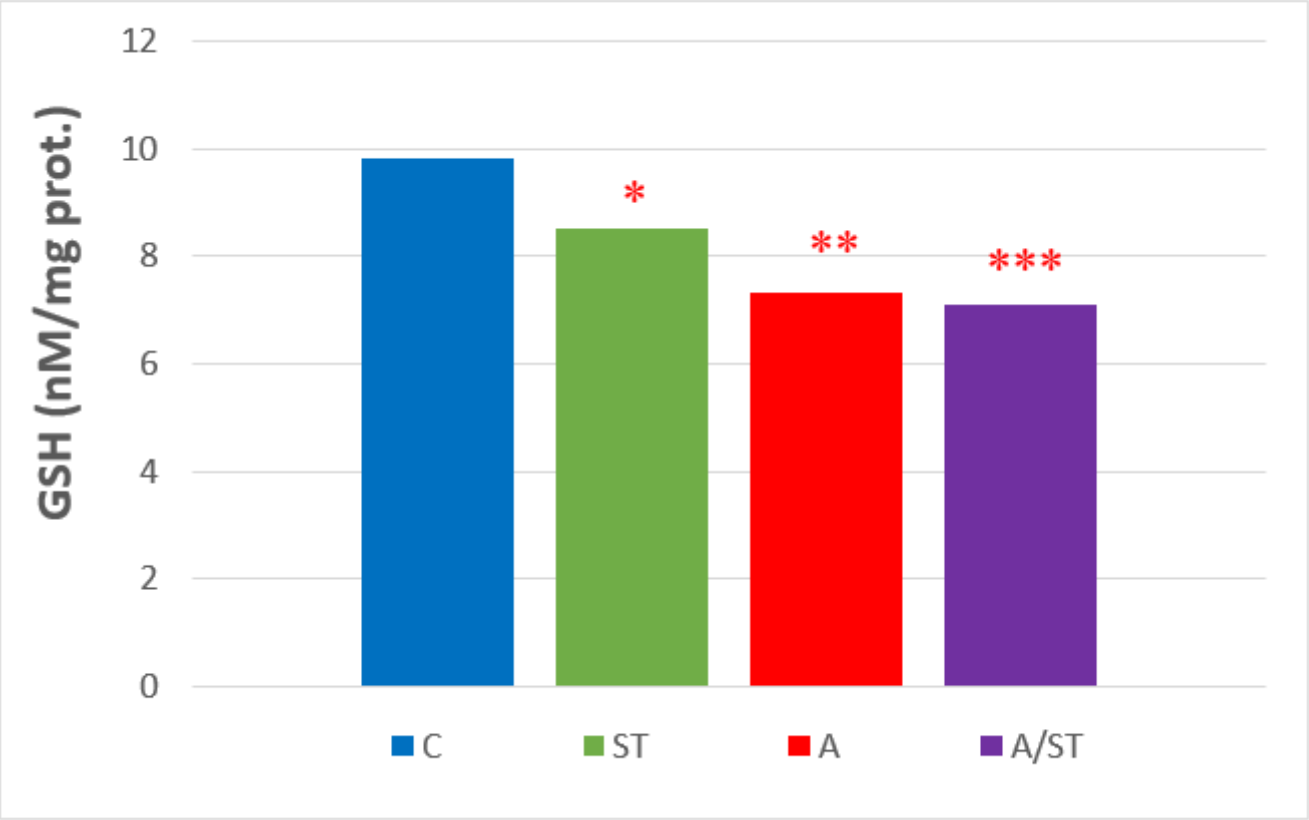

Figure 6

GSH levels (nM/mg of prot).

Significant difference: all groups compared to the control one (*p<0.05, **p<0.01, ***p<0.001).