ANTIOXIDANT RESPONSES FOLLOWING ACTIVE AND PASSIVE SMOKING OF TOBACCO AND ELECTRONIC CIGARETTES

Konstantina Poulianiti[1,2], Christina Karatzaferi[1,2], Andreas D. Flouris[1,2], Ioannis G. Fatouros[1,2], Yiannis Koutedakis[1,2,3], Athanasios Z. Jamurtas[1,2]

[1]Department of Physical Education & Sport Science, University of Thessaly, Karies, Trikala 42100, Greece.

[2]Institute of Research and Technology Thessaly, Centre for Research and Technology Hellas, Trikala, Greece.

[3]School of Sports, Performing Arts and Leisure, University of Wolverhampton, United Kingdom.

CORRESPONDING AUTHOR:

Athanasios Z. Jamurtas

Department of Physical Education & Sport Science

University of Thessaly

Karies, Trikala 42100, Greece, Tel: +30 24310 47054; Fax: +30 24310 47054

E-mail: ajamurt@pe.uth.gr

Keywords: oxidative stress; redox status; glutathione; secondhand smoke; free radicals.

ABSTRACT

Context: It has been indicated that acute active and passive tobacco cigarette smoking may cause changes on redox status balance that may result in significant pathologies. However, no study has evaluated the effects of active and passive e-cigarette smoking on redox status of consumers.

Objective: To examine the acute effects of active and passive e-cigarette and tobacco cigarette smoking on selected redox status markers.

Methods: Using a randomized single-blind crossover design, 30 participants (15 smokers and 15 non-smokers) were exposed to three different experimental conditions. Smokers underwent a control session, an active tobacco cigarette smoking session (smoked 2 cigarettes within 30-min) and an active e-cigarette smoking session (smoked a pre-determined number of puffs within 30-min using a liquid with 11 ng/ml nicotine). Similarly, non- smokers underwent a control session, a passive tobacco cigarette smoking session (exposure of 1 hour to 23±1 ppm of CO in a 60 m^3 environmental chamber) and a passive e-cigarette smoking session (exposure of 1 hour to air enriched with pre- determined number of puffs in a 60 m^3 environmental chamber). Total antioxidant capacity (TAC), catalase activity (CAT) and reduced glutathione (GSH) were assessed in participants' blood prior to, immediately after, and 1-hour post-exposure. **Results:** TAC, CAT and GSH remained similar to baseline levels immediately after and 1-hour-post exposure ($p>0.05$) in all trials. **Conclusions:** Tobacco and e-cigarette smoking exposure do not acutely alter the response of the antioxidant system, neither under active nor passive smoking conditions. Overall, there is not distinction between tobacco an e-cigarette active and passive smoking effects on specific redox status indices.

INTRODUCTION

During the past decade, a new product has been introduced in the tobacco smoking substitutes market, called electronic cigarette (e-cigarette) (Flouris and Oikonomou, 2010, Etter et al., 2011). The e-cigarette is an electronic nicotine delivery device which consists of a rechargeable battery, an evaporator and disposable filters (Flouris and Oikonomou, 2010, Etter et al., 2011). Due to lack of research on the safety of e-cigarettes, the World Health Organization called for intensified research assessing the health effects of e-cigarette use (World Health Organisation, 2010). Addressing this call, several studies have been recently conducted on e-cigarettes. Some of these studies showed that the levels of tobacco-specific toxicants in e-cigarette can be 9-450 times lower than in tobacco cigarette smoke (Goniewicz et al., 2014), supporting the role of e-cigarettes in reducing harm from tobacco smoking. However, other studies report that e-cigarette 'vaping' introduces new dangers. For example, it has been reported that a high toxicity component, diethylene glycol, was detected in the liquid of e-cigarettes, albeit in "approved" levels (Varlet et al., 2015). Additionally, substances like polycyclic aromatic hydrocarbons and carcinogenic nitrosamines have been detected in some e-cigarette liquids (Pisinger and Dossing, 2014). Overall, the presence of such toxic agents appears to be brand-specific, as researchers examining other brands found no traces of toxic or carcinogenic substances (Leondiadis, 2009). The above and other conflicting reports have prompted calls for effective regulation and promotion of e-cigarette as substitutes to tobacco smoking for existing smokers (Etter et al., 2011, Farsalinos and Le Houezec, 2015), especially given the appeal of e-cigarettes to adolescents (Dutra and Glantz, 2014).

Oxidative stress reflects an imbalance between the systemic manifestation of free radicals and the biological system's ability to detoxify these reactive intermediates or to repair the resulting damage (Sies and Cadenas, 1985). This redox imbalance may cause toxic effects that damage all components of the cell including proteins, lipids and nucleic acids. Thus, oxidative stress is thought to be involved in the

development of several diseases such as cancer, cardiovascular disease, Parkinson's disease, Alzheimer's disease and chronic fatigue syndrome (Sies and Cadenas, 1985). Reports indicate that the acute and long term (i.e., chronic) effects of active and passive tobacco cigarette smoking may attenuate the antioxidant defense system response which can, in turn, generate long-term pathologies (Valkonen and Kuusi, 1998, Scheffler et al., 1992). For example, increased lipid peroxidation and protein modification due to smoking has been reported extensively in the literature when smokers were compared to non-smokers (Pignatelli et al., 2001, Petruzzelli et al., 1997). Even though the exact mechanism that links smoking with the development of atherosclerosis and damage to the vascular wall is still unclear, evidence suggests that oxidants delivered by the tar and the gas phase become deposited in the lung and also are delivered directly to the plasma and the vasculature and therefore activating enzymes responsible for pro-oxidant development in the vascular wall (Heitzer et al., 2000). Furthermore, damage to the vasculature can occur due to activation of neutrophils, monocytes, platelets, and T cells. In addition, endothelial dysfunction that is present in active and passive smokers is augmented by the antioxidant vitamin C, indicating that oxidative stress plays a crucial role in that phenomenon (Heitzer et al., 2000).

Currently, the effects of active and passive e-cigarette smoking on redox status remain unknown. Evaluating the human redox status response to active and passive e-cigarette smoking may aid in the understanding of oxidative stress contribution to the development of smoking-related diseases. Therefore, the aim of this investigation was to examine the acute effects of active and passive e-cigarette and tobacco cigarette smoking on selected redox status markers. Based on the aforementioned findings that e-cigarettes may generate a reduced disruption in different body systems, it was hypothesized that the acute effects of active and passive e-cigarette smoking on redox status would be less pronounced as compared to active and passive tobacco cigarette smoking.

MATERIALS AND METHODS

Participants

The experimental protocol was approved by the Ethics Committee of the Department of Physical Education and Sports Science at the University of Thessaly. Two groups of healthy adult volunteers participated and signed a written consent after being informed of all risks and benefits of the study. One group consisted of 15 smokers (≥15 cigarettes / day; 8 men; 7 women; 36.8±9.9 years; BMI 25.6±4.1 kg/m^2) and another group consisted of 15 non-smokers (8 men; 7 women; 28.87±10.5 years; BMI 23.6±3.0 kg/m^2). Exclusion criteria included acute illness, cardiovascular diseases, intake of medication or antioxidant supplements, pregnancy and previous use of e-cigarette by smokers. For non-smokers, smoking was an additional exclusion criterion.

Study Design and Procedures

Participants in each group (active smokers and passive non-smokers) underwent three different experimental sessions that were performed in a random order (separated by a minimum of seven days). Participants were required to avoid food for 10 hours, strenuous physical activity for 72 hours and smoking or exposure to smoke during the night prior to the measurements. This study was part of a larger study and the procedures followed are described in detail elsewhere (Flouris et al., 2013).

Active smokers

Active smokers underwent a control session (AS_{CON}), an active tobacco cigarette smoking session (AS_{TOB}) and an active e- cigarette smoking session ($AS_{E\text{-}CIG}$). Blood samples were collected prior to, immediately after, as well as one hour after the smoking sessions. During the AS_{CON} session, participants were asked

to draw puffs on a non-lit cigarette of their own brand, for 30 minutes. In the AS_{TOB} session participants smoked two tobacco cigarettes of their own brand within 30 minutes. During the $AS_{E\text{-}CIG}$ session, participants smoked a certain number of puffs on an e-cigarette (device: GIANT, NOBACCO GP, Greece) within 30 minutes. The e-cigarette liquid (NOBACCO USA MIX, NOBACCO GP, Greece) used, had tobacco taste and contained 11 mg/ml nicotine. The specific e-cigarette and liquid was selected for the study due to the fact that this liquid was the only one in Greece that had been previously analyzed by an independent publicly-funded Research Institute and consisted of >60% propylene glycol, <10% nicotine, <5% linalool, <5% tobacco essence and <1% methyl vanilyn (Leondiadis, 2009). A previously-developed equation (Flouris et al., 2013) was used to calculate the number of e-cigarette puffs to ensure that the amount of nicotine entering the human body would be equivalent to the amount of nicotine inhaled when smoking a tobacco cigarette: [(mg of nicotine in own brand of tobacco cigarettes x 1.5 x 50)/11] x 2.

Passive non-smokers

Non-smokers underwent a control session (PS_{CON}), a passive tobacco cigarette smoking session (PS_{TOB}) and a passive e-cigarette smoking session ($PS_{E\text{-}CIG}$). Blood samples were collected prior to, immediately after, as well as one hour after the smoking sessions, as previously described (Flouris et al., 2013). Briefly, in the PS_{CON} session, participants were asked to remain for 1 hour in a 60 m^3 environmental chamber (air temperature: 21°C; air velocity: 0.05 m/sec; humidity: 45%) while breathing normal air. In the PS_{TOB} session, participants were exposed in the same chamber for 1 hour to air polluted with tobacco cigarette smoke at a CO concentration of 23±1 ppm, that is similar to the one at bar/restaurant levels (CO90 CO-CO_2 analyzer, Martindale Electric Ltd., Watford, UK). The required concentration of the gas mixture was achieved by combustion of cigarettes from several popular brands using an air pump

(DYN, Volos, Greece), which regulated the airflow at 4 L/min. Cigarettes were half smoked using the air pump, then they were left lit for 2 min to generate sidestream smoke, and then the rest of the cigarettes were smoked via the air pump. An average of 29.2±0.9 cigarettes was smoked in order to achieve the required level of CO in the exposure chamber. In the $PS_{E\text{-}CIG}$ session, participants were exposed to air polluted with e-cigarette vapour for one hour in the same chamber. In this case, the air was polluted by smoking e-cigarettes (device and liquid same as those used during the $AS_{E\text{-}CIG}$ session) via the same air pump set at an air flow rate of 4 L/min for the same time as in the PS_{TOB} session.

Blood Collection and Handling

Blood samples (5 ml) were collected from each antecubital vein and were placed into EDTA-containing tubes (20 µl ETDA/ml of blood). In order to obtain plasma, samples were centrifuged immediately at 1,370 x g, at 4°C for 10 min. The supernatant plasma was collected, aliquoted in Eppendorf tubes, stored at -80°C and thawed only once before analyzed for total antioxidant capacity (TAC). Red blood cells lysate was produced by diluting packed erythrocytes with distilled water (1:1 vol:vol). The mixture was inverted vigorously and centrifuged at 4,000 x g, at 4°C for 15 min. The supernatant was collected and aliquoted in Eppendorf tubes, stored at -80°C and thawed only once before analyzed for reduced glutathione (GSH) and catalase activity (CAT). Finally, in order to obtain serum, another portion of blood sample (5 ml) was collected and placed into separate tubes containing clot activator, left for 20 min to clot at room temperature, and centrifuged at 1,370 x g, at 4°C for 10 min. The supernatant was collected and aliquoted in Eppendorf tubes, stored at -80°C and thawed only once before analyzed for cotinine.

Assays

Procedures used to assess cotinine levels in circulating blood are described elsewhere (Flouris et al., 2013). Reduced glutathione concentration was determined using previous methodology (Reddy et al., 2004). Briefly, 500 µl of red blood cells lysate was treated with 500 µl TCA 5%, centrifuged at 16000 x g, at 4°C for 10 min. 300 µl of the supernatant was collected and added in 90 µl TCA 5%, centrifuged at 16000 x g, at 4°C for 10 min. Thereafter, 20 µl of the TCA treated supernatant was mixed with 660 µl of 67 mM sodium-potassium phosphate (pH 7.95) and 330 µl of 1 mM 5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB). The samples were incubated in the dark at room temperature for 45 min and the absorbance was read at 412 nm using a spectrophotometer. The GSH concentration was calculated by standard curve constructed using commercially available standards.

Catalase activity was measured according to the Aebi assay (Aebi, 1984). Briefly, 4 µl of red blood cells lysate (diluted 1:10) were added to 2,991 µl of 67 mM sodium-potassium phosphate buffer (pH 7.4) and the samples were incubated at 37°C for 10 min. The sample was transferred into a glass cuvette, 5µl of 30% hydrogen peroxide was added and the change in absorbance was immediately read at 240 nm for 90 sec.

Total Antioxidant Capacity was determined according to Janaszewska & Bartosz assay (Janaszewska and Bartosz, 2002), based on the scavenging of 2,2-diphenyl-1 picrylhydrazyl (DPPH) free radical. DPPH stock solution (10mM) was prepared by dissolving 0.02g DPPH in 5ml of methanol and mix in the stirrer. The working solution was obtained by diluting stock solution 100 times with methanol. Afterwards, 20µl of plasma was mixed with 480µl of 10mM sodium-potassium phosphate (pH 7.4) and 500µl of 0.1 M working solution (DPPH). The samples were incubated in the dark for 30 min at room temperature, centrifuged at 20,000 x g, at 25°C for 3 min. 900 µl of the supernatant was transferred into a clean plastic cuvette and the absorbance was read at 520 nm using a spectrophotometer. TAC is presented as mM of DPPH reduced to 2,2-diphenyl-1 picrylhydrazine (DPPH:H).

Intra-assay correlation coefficient for GSH, CAT and TAC were 3.5%, 2.8% and 2.2%, respectively.

Statistical Analysis

Based on data from a previously-published study (Flouris, 2009), the resulting minimum required sample size for this study was eight participants per group, providing a reliability rate of 95%. To reduce the probability of making a type II error, 15 participants were recruited for each group (smokers and non-smokers). Normality was assessed by the Kolmogorov–Smirnov test and the distribution of all variables was not found significantly different from normal. A two-way (trial × time) ANOVA with repeated measures of time was used to assess differences between the three conditions in smokers and non-smokers. Furthermore, a two way (condition x time) ANOVA was used to assess differences between the passive and active condition. The level of statistical significance was set at $p<0.05$. Data are presented as mean±SD. The SPSS version 15.0 (SPSS Inc., USA) was used for all analyses.

RESULTS

Active Smoking

No changes were observed in GSH (Figure 2A), CAT (Figure 3A), and TAC (Figure 4A) concentrations prior to, immediately after and 1-hour after the exposure to AS_{TOB}, $AS_{E\text{-}CIG}$ and AS_{CON} ($p>0.05$). Furthermore, no interaction between the three conditions was revealed for any time point of assessment in any of the three variables (GSH, CAT, TAC) assessed ($p>0.05$). Specifically, GSH (μmol/g Hb) levels for AS_{TOB} were 2.21±0.85 prior to, 2.11±0.76 immediately after, and 1.72±0.82 one hour after the smoking sessions, respectively. During $AS_{E\text{-}CIG}$, GSH levels were 2.07±1.25 prior to, 2.17±0.87 immediately after, and 2.26±0.86 one hour after the smoking sessions, respectively. Finally, GSH levels for AS_{CON} were 1.65±0.87 prior to, 1.80±1.06 immediately after, and 1.92±1.28 one hour after the smoking sessions, respectively.

CAT (µmol/min/mg Hb) activity for AS_{TOB} was 347±33 prior to, 332±14 immediately after, and 389±43 one hour after the smoking sessions, respectively. Also, CAT activity for $AS_{E\text{-}CIG}$ was 357±14 prior to, 331±55 immediately after, and 354±29 one hour after the smoking sessions, respectively. Finally, CAT activity for AS_{CON} was 338±53 prior to, 349±66 immediately after, and 329±51 one hour after the smoking sessions, respectively. TAC (mM DPPH) levels for AS_{TOB} were 1.03±0.09 prior to, 1.01±0.09 immediately after and 0.99±0.09 one hour after the smoking sessions, respectively. During $AS_{E\text{-}CIG}$, TAC levels were 0.98±0.14 prior to, 0.96±0.11 immediately after, and 0.95±0.12 one hour after the smoking sessions, respectively. Finally, TAC levels for AS_{CON} were 1.04±0.08 prior to, 1.00±0.10 immediately after, and 1.01±0.09 one hour after the smoking sessions, respectively.

Passive Smoking

No time-dependent changes were found in GSH (Figure 2B), CAT (Figure 3B), and TAC (Figure 4B) concentration prior to, immediately after and one hour after the exposure to PS_{TOB}, $PS_{E\text{-}CIG}$ and PS_{CON} ($p>0.05$). No interaction between the three conditions was revealed for any time point of assessment in any of the three variables (GSH, CAT, TAC) assessed ($p>0.05$). Specifically, GSH (µmol/g Hb) levels for PS_{TOB} were 2.08±1.02 prior to, 1.65±0.66 immediately after, and 1.53±0.81 one hour after the smoking sessions, respectively. During $PS_{E\text{-}CIG}$ GSH levels were 2.35±1.20 prior to, 2.65±0.81 immediately after, and 2.78±1.18 one hour after the smoking sessions, respectively. Finally, GSH levels for PS_{CON} were 1.68±0.85 prior to, 1.42±0.97 immediately after, and 1.51±0.94 one hour after the smoking sessions, respectively. CAT (µmol/min/mgHb) activity for PS_{TOB} was 385±53 prior to, 382±45 immediately after, and 377±41 one hour after the smoking sessions, respectively. Also, CAT activity for $PS_{E\text{-}CIG}$ was 331±60 prior to, 326±50 immediately after, and 354±72 one hour after the smoking sessions, respectively. Finally, CAT activity for PS_{CON} was 357±90 prior to, 330±56 immediately after, and 327±60 one hour after

the smoking sessions, respectively. TAC (mM DPPH) levels for PS_{TOB} were 1.03±0.16 prior to, 1.04±0.17 immediately after and 1.02±0.16 one hour after the smoking sessions, respectively. During $PS_{E\text{-}CIG}$, TAC levels were 1.03±0.16 prior to, 1.06±0.18 immediately after, and 1.04±0.13 one hour after the smoking sessions, respectively. Finally, TAC levels for PS_{CON} were 1.00±0.18 prior to, 0.97±0.14 immediately after, and 0.99±0.17 one hour after the smoking sessions, respectively.

DISCUSSION

To our knowledge, this is the first study to examine the acute effects of active and passive e-cigarette smoking on the antioxidant response in human blood. The results indicate that active and passive smoking of e-cigarettes and tobacco cigarettes does not result in immediately visible perturbations of the blood antioxidant system as evaluated by GSH, CAT and TAC.

Active Smoking

Previous research indicates that active smoking may have detrimental effects on human health (Flouris et al., 2010). Tobacco smoking accounts for the largest amount of deaths and disability years in high-income countries and is the third leading cause of death and disabilities in developing countries (Lopez et al., 2006). Tobacco contains several substances that may influence the human physiology and has been linked to oxidative stress development (Halliwell and Poulsen, 2006). Since the number of deaths is mounting and the economic burden is escalating in the recent years, new products have been introduced in the market to counteract the negative effects of smoking. Electronic cigarette is one of them and some studies indicate that cotinine levels are similar to the one found after tobacco smoking (Flouris et al., 2013).

Smoking either e-cigarettes or conventional tobacco cigarettes may disrupt human health. A recent *in vitro* study showed that tobacco and e-cigarette products may initiate an inflammatory response and oxidative stress production in Kupffer cells in the liver (Rubenstein et al., 2015). Furthermore, short-term e-cigarette "vaping" generates smaller changes in lung function compared to tobacco cigarette smoking (Flouris et al., 2013, Vardavas et al., 2011). However, inhaling an e-cigarette for 5 min results in attenuated exhaled nitric oxide levels (Vardavas et al., 2011, Marini et al., 2014). Changes in peripheral flow resistance due to acute narrowing of the diameter of the peripheral pathways and lower levels of nitric oxide have been proposed as possible mechanisms for the changes in exhaled nitric oxide levels following either tobacco or e-cigarette smoking (Vardavas et al., 2011). Nitric oxide, besides having an important role in several physiological processes of the respiratory tract, has been used as a marker for assessing oxidative stress (Hoyt et al., 2003).

In the literature, several studies have assessed the antioxidant status of healthy smokers. According to Moriarty et al (Moriarty et al., 2003) a significant decrease in GSH levels in plasma of smokers compared to non smokers, was found. However, regarding the activities of three of the most significant antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidise (GPx) and CAT, contradictory results were found. Another study (Zhou et al., 2000) observed decreased activity of these enzymes in erythrocytes of smokers compared to non smokers. Yildiz et al (Yildiz et al., 2002), found decreased CAT and SOD activities, however, GPx activity remained unchanged in erythrocytes of smokers compared to non smokers, On the other hand, increased activities of these enzymes were observed in the erythrocytes of young smokers compared to age-matched non smokers (Ozguner et al., 2005), reflecting that the small duration of smoking perhaps was not efficient to alter their enzymatic antioxidant response. Finally, Meta et al (Metta et al., 2015), assessed the erythrocyte antioxidant defense against cigarette smoking, finding that there was highly significant difference in SOD activity, in

comparison to CAT and GPx between smokers and non smokers. Indeed, while CAT and GPx activities were found decreased, SOD activity was found increased in smokers compared to non smokers. TAC was also assessed in blood of smokers and non smokers and was found unchanged in the study of Kurku et al (Kurku et al., 2015) and decreased in smokers in the study of Mojtaba et al (Mojtaba et al., 2014)

In the study of Kurku et al (Kurku et al., 2015) acute effects of smoking on antioxidant defense were also assessed. Specifically, saliva samples were collected from smokers 1 h after smoking only one cigarette. Nevertheless, no difference in the levels of TAC, SOD and GPx was observed pre and before smoking. The short period of exposure to only one cigarette, was not enough to cause antioxidant defense modifications. The aforementioned attenuation of exhaled nitric oxide levels following active e-cigarette and tobacco cigarette smoking may suggest changes in redox status. However, we did not observe any statistically significant acute changes in antioxidant levels either in time or between groups. We scrutinized our data further by examining our data as percentage of initial value, to account for subject variability (data not shown) without again finding statistically significant alterations in antioxidant levels. Perhaps the oxidative stress does not stem from a reduction in antioxidant capacity but from an increase in oxidants. Moreover, the tissue assessed may play a role. In this study we assessed changes in the antioxidant system in blood and red blood cells lysate (to systemic oxidative status) whereas the exhaled nitric oxide levels assessed in previous studies indicates airway oxidative stress. Additionally, we restricted our study to the immediate effects of smoking on antioxidant levels. Therefore, we cannot exclude that reductions in antioxidant capacity could be revealed if sampling was repeated some hours after the exposure. Further studies are needed to elucidate possible differences in the redox response between tissues, especially following active e-cigarette smoking.

Passive Smoking

Chronic exposure to passive tobacco smoking has been shown to generate unfavourable health effects (Faught et al., 2009, Carrillo et al., 2009) since it increases the risk of heart disease by approximately 30% whereas acute exposure (minutes to hours) has nearly as large effects as chronic active smoking (Metsios et al., 2007, Dinas et al., 2011, Barnoya and Glantz, 2005). The negative health effects of tobacco passive smoking are exemplified by acute unfavorable effects on lung function, cytokine production, thyroid hormone secretion, and complete blood count parameters (Dinas et al., 2014, Flouris et al., 2008, Metsios et al., 2007). Chronic exposure to passive tobacco smoking has been also shown to result in changes in redox balance. Exposure to chronic passive smoking results in a 63% increase in 8-hydroxy-2'-deoxyguanosine in the blood of exposed subjects and 13% and 37% increase in catalase and glutathione peroxidase activity, respectively (Howard et al., 1998). Furthermore, a strong association between serum protein carbonyl and malondialdehyde concentration has been revealed in non-smoker patients hospitalized for acute myocardial infarction (Megson et al., 2013). Yildiz et al (Yildiz et al., 2002), evaluated the erythrocyte antioxidant defence of passive smokers and found significantly decreased CAT and SOD activities in passive smokers in comparison to non smokers. Based on these findings, it is clear that exposure of chronic passive tobacco smoking can lead to changes in redox status. However, there is only a limited number of interventional studies that assessed oxidative stress responses following a single exposure to passive smoking. These studies indicate that acute passive tobacco smoking results in airway acidification, oxidative stress, and decreased levels of FeNO (Flouris et al., 2013, Kostikas et al., 2013). Furthermore, significant increases have been reported in 8-isoprostane levels after 30 min of passive smoking, an increase that was equivalently to the levels of smokers (Kato et al., 2006). These results are in contrast with the current findings of no changes in redox status markers neither immediately post nor 1-hour post exposure. Possible reasons for this contradiction might be that we assessed components of the antioxidant system whereas the previous studies assessed

markers of oxidative stress. Furthermore, two of the three aforementioned studies assessed oxidative stress in lungs. We assessed systemic oxidative stress in molecules found in the blood which may not reflect lung tissue levels, or may require some time before any changes are observed.

Acute exposure to passive e-cigarette environments has been shown to increase cotinine levels to an extent similar to that of passive tobacco cigarette smoking (Flouris et al., 2013). However, passive e-cigarette vapour exposure shows no significant changes in lung function (Flouris et al., 2013). Furthermore, passive e-cigarette vapour exposure does not appear to influence the results of the complete blood count examination (Flouris et al., 2012). The present results indicate that a 1-hour exposure to a passive e-cigarette environment does not cause significant perturbations in the antioxidant system up to 1-hour post-exposure. It is possible that changes would become evident if we had continued our observation for a longer period. Finally, as our measurements indicate systemic redox status, we cannot exclude alterations at the level of the lung epithelial cells, as observed in a mouse study (Lerner et al., 2015).

It is important to note that, while we instructed our subjects to consume similar meals in the day prior to each assessment, we did not strictly monitor their diet. High levels of food and/or high activity of other endogenous antioxidants may explain the lack of responses in the antioxidant system following the active or passive smoking procedures. This is because the bioavailability of the antioxidant vitamin C changes following smoking whereas its turnover in smokers is 40% higher compared to non-smokers (German Nutrition, 2015).

CONCLUSION

In conclusion, we found that tobacco and e-cigarette smoking exposure do not acutely alter the response of the antioxidant system, neither under active nor passive smoking conditions. Overall, there

is not distinction between tobacco and e-cigarette active and passive smoking effects on specific redox status indices.

REFERENCES

AEBI, H. 1984. Catalase in vitro. *Methods Enzymol,* 105**,** 121-6.

BARNOYA, J. & GLANTZ, S. A. 2005. Cardiovascular effects of secondhand smoke: nearly as large as smoking. *Circulation,* 111**,** 2684-98.

CARRILLO, A. E., METSIOS, G. S. & FLOURIS, A. D. 2009. Effects of secondhand smoke on thyroid function. *Inflamm Allergy Drug Targets,* 8.

DINAS, P. C., KOUTEDAKIS, Y. & FLOURIS, A. D. 2011. Effects of active and passive tobacco cigarette smoking on heart rate variability. *Int J Cardiol,* 163**,** 109-115.

DINAS, P. C., METSIOS, G. S., JAMURTAS, A. Z., TZATZARAKIS, M. N., WALLACE HAYES, A., KOUTEDAKIS, Y., TSATSAKIS, A. M. & FLOURIS, A. D. 2014. Acute effects of second-hand smoke on complete blood count. *Int J Environ Health Res,* 24**,** 56-62.

DUTRA, L. M. & GLANTZ, S. A. 2014. Electronic cigarettes and conventional cigarette use among U.S. adolescents: a cross-sectional study. *JAMA Pediatr,* 168**,** 610-7.

ETTER, J. F., BULLEN, C., FLOURIS, A. D., LAUGESEN, M. & EISSENBERG, T. 2011. Electronic nicotine delivery systems: a research agenda. *Tob Control,* 20**,** 243-8.

FARSALINOS, K. E. & LE HOUEZEC, J. 2015. Regulation in the face of uncertainty: the evidence on electronic nicotine delivery systems (e-cigarettes). *Risk Manag Healthc Policy,* 8**,** 157-67.

FAUGHT, B. E., FLOURIS, A. D. & CAIRNEY, J. 2009. Epidemiological evidence associating secondhand smoke exposure with cardiovascular disease. *Inflamm Allergy Drug Targets,* 8.

FLOURIS, A. D., CHORTI, M. S., POULIANITI, K. P., JAMURTAS, A. Z., KOSTIKAS, K., TZATZARAKIS, M. N., WALLACE HAYES, A., TSATSAKIS, A. M. & KOUTEDAKIS, Y. 2013. Acute impact of active and passive electronic cigarette smoking on serum cotinine and lung function. *Inhal Toxicol,* 25**,** 91-101.

FLOURIS, A. D., METSIOS, G. S., JAMURTAS, A. Z. & KOUTEDAKIS, Y. 2008. Sexual dimorphism in the acute effects of secondhand smoke on thyroid hormone secretion, inflammatory markers and vascular function. *Am J Physiol Endocrinol Metab,* 294**,** E456-62.

FLOURIS, A. D., METSIOS, G. S., JAMURTAS, A. Z. & KOUTEDAKIS, Y. 2010. Cardiorespiratory and immune response to physical activity following exposure to a typical smoking environment. *Heart,* 96**,** 860-4.

FLOURIS, A. D. & OIKONOMOU, D. N. 2010. Electronic cigarettes: miracle or menace? *BMJ,* 340**,** c311.

FLOURIS, A. D., POULIANITI, K. P., CHORTI, M. S., JAMURTAS, A. Z., KOURETAS, D., OWOLABI, E. O., TZATZARAKIS, M. N., TSATSAKIS, A. M. & KOUTEDAKIS, Y. 2012. Acute effects of electronic and tobacco cigarette smoking on complete blood count. *Food Chem Toxicol,* 50**,** 3600-3603.

GERMAN NUTRITION, S. 2015. New Reference Values for Vitamin C Intake. *Ann Nutr Metab,* 67**,** 13-20.

GONIEWICZ, M. L., KNYSAK, J., GAWRON, M., KOSMIDER, L., SOBCZAK, A., KUREK, J., PROKOPOWICZ, A., JABLONSKA-CZAPLA, M., ROSIK-DULEWSKA, C., HAVEL, C., JACOB, P., 3RD & BENOWITZ, N. 2014. Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. *Tob Control,* 23**,** 133-9.

HALLIWELL, B. B. & POULSEN, H. E. 2006. *Cigarette smoke and oxidative stress,* Berlin, Germany, Springer-Verlag.

HEITZER, T., BROCKHOFF, C., MAYER, B., WARNHOLTZ, A., MOLLNAU, H., HENNE, S., MEINERTZ, T. & MUNZEL, T. 2000. Tetrahydrobiopterin improves endothelium-dependent vasodilation in chronic smokers : evidence for a dysfunctional nitric oxide synthase. *Circ Res,* 86**,** E36-41.

HOWARD, D. J., BRIGGS, L. A. & PRITSOS, C. A. 1998. Oxidative DNA damage in mouse heart, liver, and lung tissue due to acute side-stream tobacco smoke exposure. *Arch Biochem Biophys,* 352**,** 293-7.

HOYT, J. C., ROBBINS, R. A., HABIB, M., SPRINGALL, D. R., BUTTERY, L. D., POLAK, J. M. & BARNES, P. J. 2003. Cigarette smoke decreases inducible nitric oxide synthase in lung epithelial cells. *Exp Lung Res,* 29**,** 17-28.

JANASZEWSKA, A. & BARTOSZ, G. 2002. Assay of total antioxidant capacity: comparison of four methods as applied to human blood plasma. *Scand J Clin Lab Invest,* 62**,** 231-6.

KATO, T., INOUE, T., MOROOKA, T., YOSHIMOTO, N. & NODE, K. 2006. Short-term passive smoking causes endothelial dysfunction via oxidative stress in nonsmokers. *Can J Physiol Pharmacol,* 84**,** 523-9.

KOSTIKAS, K., MINAS, M., NIKOLAOU, E., PAPAIOANNOU, A. I., LIAKOS, P., GOUGOURA, S., GOURGOULIANIS, K. I., DINAS, P. C., METSIOS, G. S., JAMURTAS, A. Z., FLOURIS, A. D. & KOUTEDAKIS, Y. 2013. Secondhand smoke exposure induces acutely airway acidification and oxidative stress. *Respir Med,* 107**,** 172-9.

KURKU, H., KACMAZ, M., KISA, U., DOGAN, O. & CAGLAYAN, O. 2015. Acute and chronic impact of smoking on salivary and serum total antioxidant capacity. *J Pak Med Assoc,* 65**,** 164-9.

LEONDIADIS, L. 2009. *Results of chemical analyses in NOBACCO electronic cigarette refills* [Online]. Athens, Greece: Mass Spectrometry and Dioxin Analysis Lab, National Center for Scientific Research "Demokritos". Available: http://www.nobacco.gr/datafiles/files/DIMOKRITOS.pdf [Accessed October 19 2009].

LERNER, C. A., SUNDAR, I. K., YAO, H., GERLOFF, J., OSSIP, D. J., MCINTOSH, S., ROBINSON, R. & RAHMAN, I. 2015. Vapors produced by electronic cigarettes and e-juices with flavorings induce toxicity, oxidative stress, and inflammatory response in lung epithelial cells and in mouse lung. *PLoS One,* 10**,** e0116732.

LOPEZ, A. D., MATHERS, C. D., EZZATI, M., JAMISON, D. T. & MURRAY, C. J. L. 2006. *Global burden of disease and risk factors,* New York, USA, The World Bank and Oxford University Press.

MARINI, S., BUONANNO, G., STABILE, L. & FICCO, G. 2014. Short-term effects of electronic and tobacco cigarettes on exhaled nitric oxide. *Toxicol Appl Pharmacol,* 278**,** 9-15.

MEGSON, I. L., HAW, S. J., NEWBY, D. E. & PELL, J. P. 2013. Association between exposure to environmental tobacco smoke and biomarkers of oxidative stress among patients hospitalised with acute myocardial infarction. *PLoS One,* 8**,** e81209.

METSIOS, G. S., FLOURIS, A. D., JAMURTAS, A. Z., CARRILLO, A. E., KOURETAS, D., GERMENIS, A. E., GOURGOULIANIS, K., KIROPOULOS, T., TZATZARAKIS, M. N., TSATSAKIS, A. M. & KOUTEDAKIS, Y. 2007. A brief exposure to moderate passive smoke increases metabolism and thyroid hormone secretion. *J Clin Endocrinol Metab,* 92**,** 208-11.

METTA, S., BASALINGAPPA, D. R., UPPALA, S. & MITTA, G. 2015. Erythrocyte Antioxidant Defenses Against Cigarette Smoking in Ischemic Heart Disease. *J Clin Diagn Res,* 9**,** BC08-11.

MOJTABA, W., DAVOOD, K. & HUSSEIN, D. 2014. Lower Total Antioxidant Capacity in Smokers Compare to

Non-smokers. *Biological Forum – An International Journal,* 6**,** 305-309.

MORIARTY, S. E., SHAH, J. H., LYNN, M., JIANG, S., OPENO, K., JONES, D. P. & STERNBERG, P. 2003. Oxidation of glutathione and cysteine in human plasma associated with smoking. *Free Radic Biol Med,* 35**,** 1582-8.

OZGUNER, F., KOYU, A. & CESUR, G. 2005. Active smoking causes oxidative stress and decreases blood melatonin levels. *Toxicol Ind Health,* 21**,** 21-6.

PETRUZZELLI, S., PUNTONI, R., MIMOTTI, P., PULERA, N., BALIVA, F., FORNAI, E. & GIUNTINI, C. 1997. Plasma 3-nitrotyrosine in cigarette smokers. *Am J Respir Crit Care Med,* 156**,** 1902-7.

PIGNATELLI, B., LI, C. Q., BOFFETTA, P., CHEN, Q., AHRENS, W., NYBERG, F., MUKERIA, A., BRUSKE-HOHLFELD, I., FORTES, C., CONSTANTINESCU, V., ISCHIROPOULOS, H. & OHSHIMA, H. 2001. Nitrated and oxidized plasma proteins in smokers and lung cancer patients. *Cancer Res,* 61**,** 778-84.

PISINGER, C. & DOSSING, M. 2014. A systematic review of health effects of electronic cigarettes. *Prev Med,* 69**,** 248-60.

REDDY, Y. N., MURTHY, S. V., KRISHNA, D. R. & PRABHAKAR, M. C. 2004. Role of free radicals and antioxidants in tuberculosis patients. *Ind J Tuberculosis,* 51**,** 213-218.

RUBENSTEIN, D. A., HOM, S., GHEBREHIWET, B. & YIN, W. 2015. Tobacco and e-cigarette products initiate Kupffer cell inflammatory responses. *Mol Immunol,* 67**,** 652-60.

SCHEFFLER, E., WIEST, E., WOEHRLE, J., OTTO, I., SCHULZ, I., HUBER, L., ZIEGLER, R. & DRESEL, H. A. 1992. Smoking influences the atherogenic potential of low-density lipoprotein. *Clin Investig,* 70**,** 263-8.

SIES, H. & CADENAS, E. 1985. Oxidative stress: damage to intact cells and organs. *Philos Trans R Soc Lond B Biol Sci,* 311**,** 617-31.

VALKONEN, M. & KUUSI, T. 1998. Passive smoking induces atherogenic changes in low-density lipoprotein. *Circulation,* 97**,** 2012-6.

VARDAVAS, C. I., ANAGNOSTOPOULOS, N., KOUGIAS, M., EVANGELOPOULOU, V., CONNOLLY, G. N. & BEHRAKIS, P. K. 2011. Acute pulmonary effects of using an e-cigarette: impact on respiratory flow resistance, impedance and exhaled nitric oxide. *Chest*.

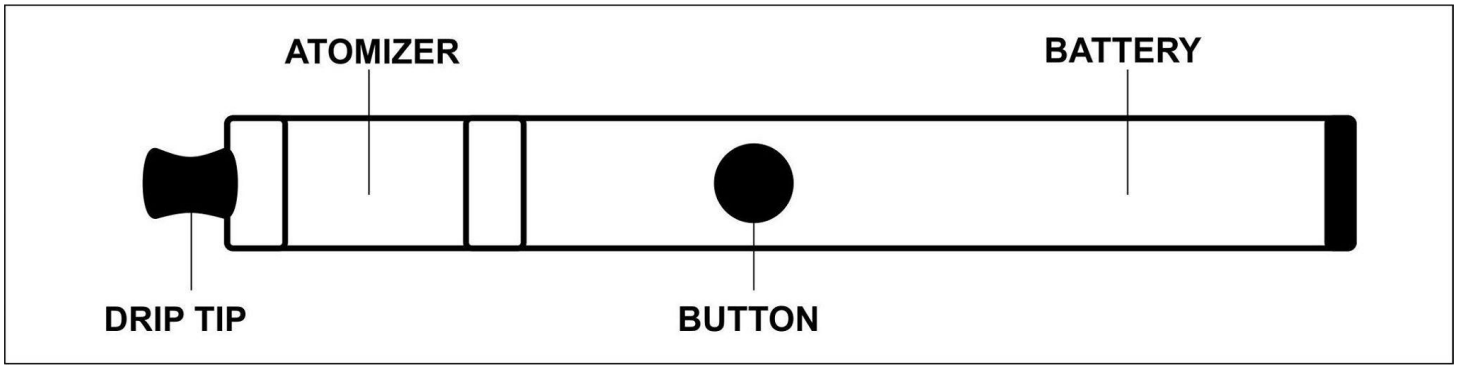
VARLET, V., FARSALINOS, K., AUGSBURGER, M., THOMAS, A. & ETTER, J. F. 2015. Toxicity assessment of refill liquids for electronic cigarettes. *Int J Environ Res Public Health,* 12**,** 4796-815.

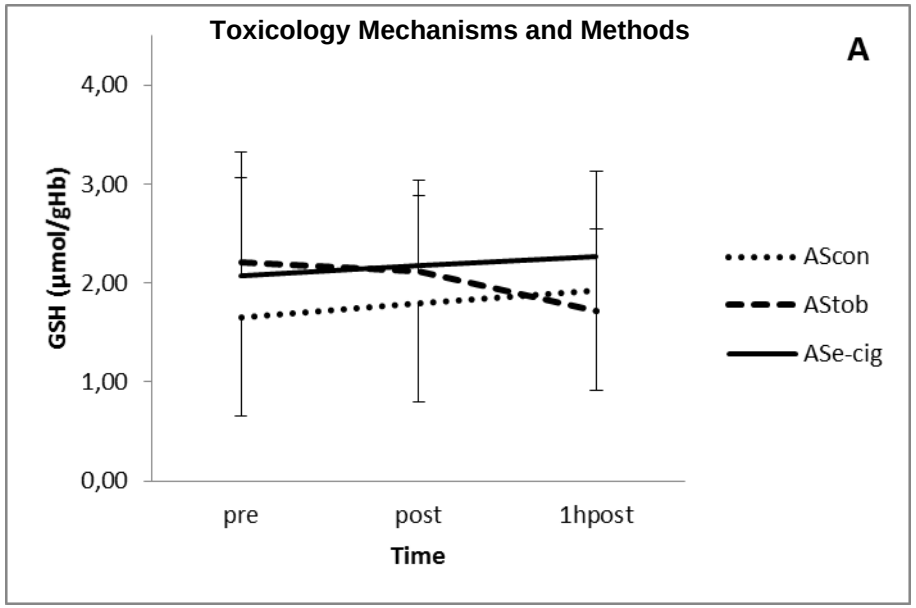
WORLD HEALTH ORGANISATION 2010. WHO Regulatory Consultation on the Safety of Electronic Nicotine Delivery Devices (ENDS). Geneva: World Health Organisation.

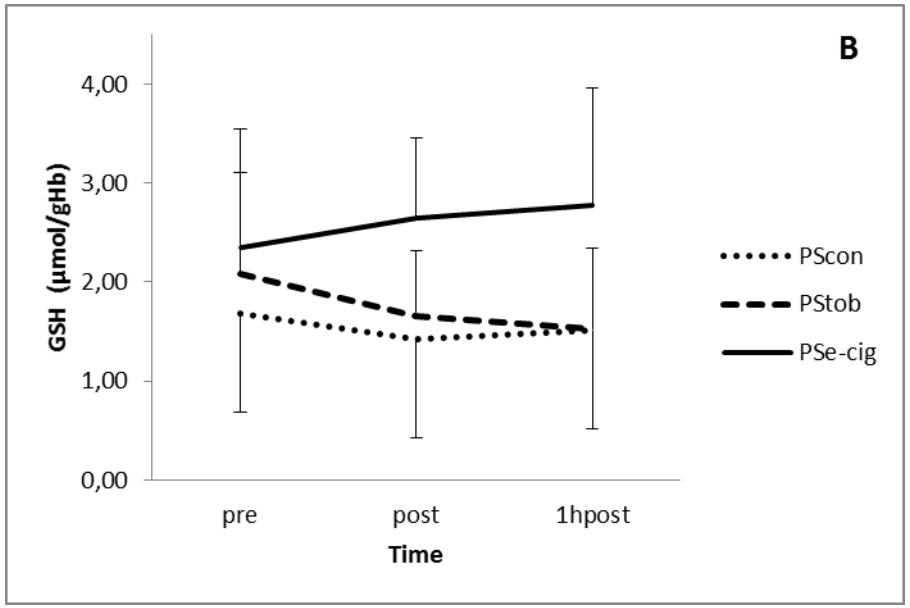
YILDIZ, L., KAYAOGLU, N. & AKSOY, H. 2002. The changes of superoxide dismutase, catalase and glutathione peroxidase activities in erythrocytes of active and passive smokers. *Clin Chem Lab Med,* 40**,** 612-5.

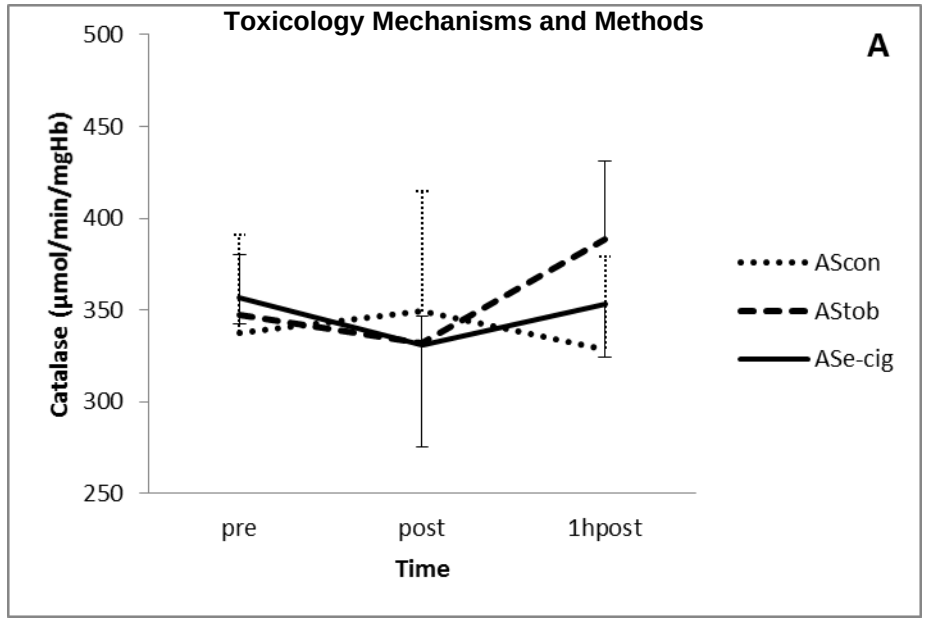
ZHOU, J. F., YAN, X. F., GUO, F. Z., SUN, N. Y., QIAN, Z. J. & DING, D. Y. 2000. Effects of cigarette smoking and smoking cessation on plasma constituents and enzyme activities related to oxidative stress. *Biomed Environ Sci,* 13**,** 44-55.

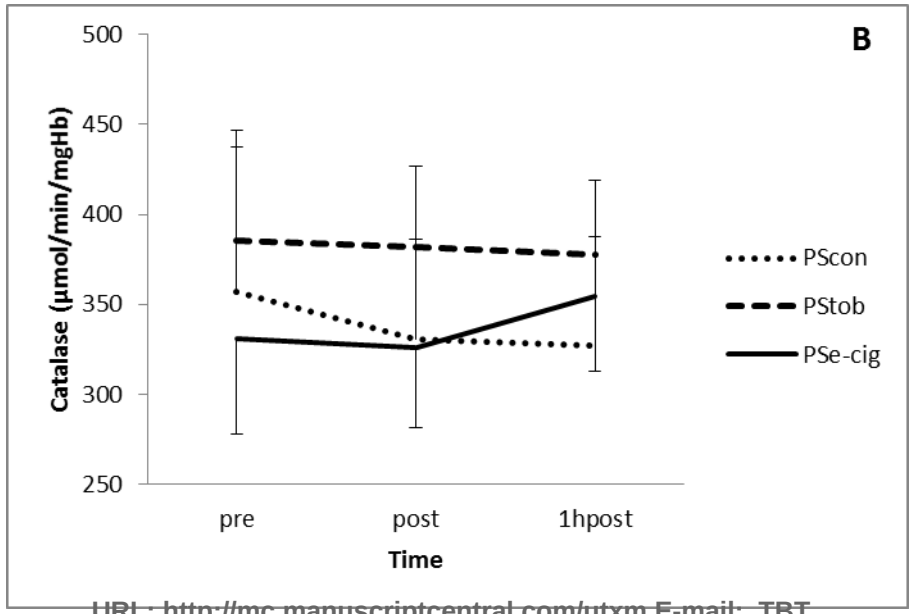
LIST OF FIGURES

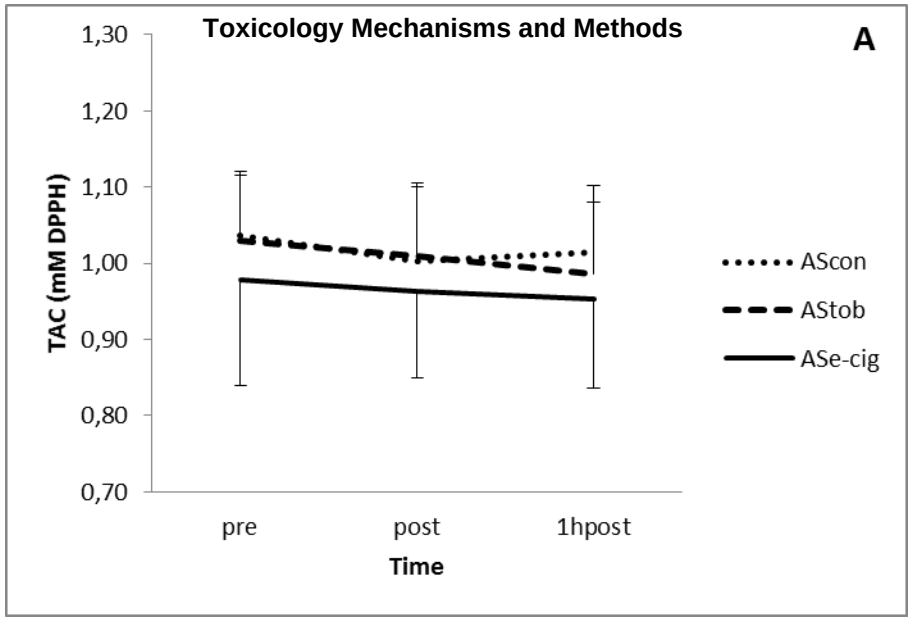

ATOMIZER
BATTERY
DRIP TIP
BUTTON

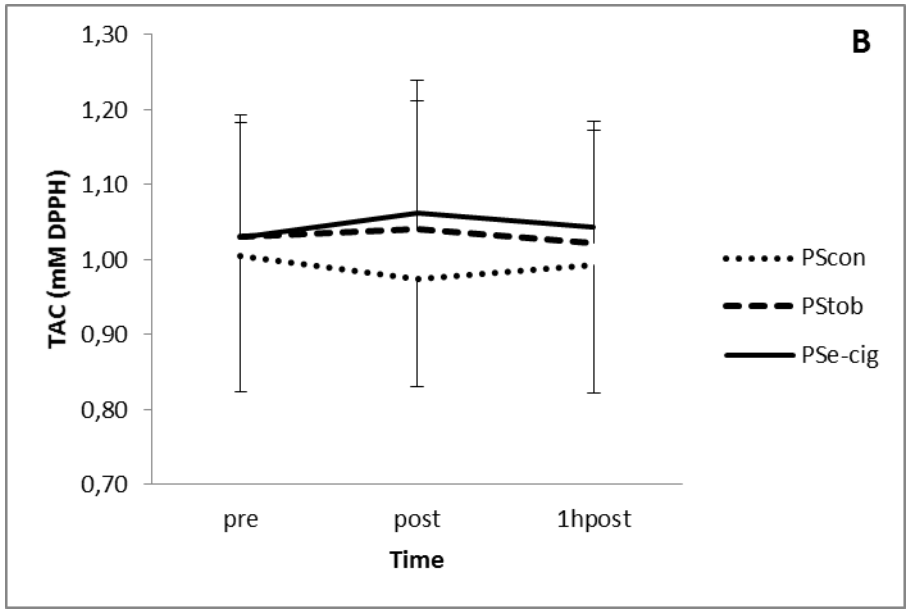

A
4,00
3,00
2,00
1,00
0,00
GSH (µmol/gHb)
pre
post
1hpost
Time
AScon
AStob
ASe-cig


B
4,00
3,00
2,00
1,00
0,00
GSH (µmol/gHb)
pre
post
1hpost
Time
PScon
PStob
PSe-cig


A
500
450
400
350
300
250
Catalase (µmol/min/mgHb)
pre
post
1hpost
Time
AScon
AStob
ASe-cig


B
500
450
400
350
300
250
Catalase (µmol/min/mgHb)
pre
post
1hpost
Time
PScon
PStob
PSe-cig


A
1,30
1,20
1,10
1,00
0,90
0,80
0,70
TAC (mM DPPH)
pre
post
1hpost
Time
AScon
AStob
ASe-cig


B
1,30
1,20
1,10
1,00
0,90
0,80
0,70
TAC (mM DPPH)
pre
post
1hpost
Time
PScon
PStob
PSe-cig