Experimed 2023; 13(2): 127-132

ORIGINAL ARTICLE

DOI: 10.26650/experimed.1319722

Comparative Toxicity Responses of Thirdhand Smoke Derived from Conventional Cigarette and Heated Tobacco Products in Human Bronchial Epithelial Cells

Rengin Reis[1,2], Kübra Kolci[1]

[1]Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Acıbadem Mehmet Ali Aydınlar University, Istanbul, Turkiye
[2]Department of Toxicology, Faculty of Pharmacy, Yeditepe University, Istanbul, Turkiye

ORCID ID: K.K. 0000-0003-4228-6564; R.R. 0000-0002-3484-2201

Cite this article as: Reis R, Kolci K. Comparative toxicity responses of thirdhand smoke derived from conventional cigarette and heated tobacco products in human bronchial epithelial cells. Experimed 2023; 13(2): 127-132.

ABSTRACT

Objective: Thirdhand smoke (THS) is described as the accumulated chemicals left on indoor surfaces after tobacco smoking. Individuals can be exposed to THS by dermal or oral contact with THS-embedded surfaces or by breathing in the off-gasses. In the present study, the cytotoxic, oxidative, and inflammatory responses of THS extracts yielded from conventional (THS-C) and heated-tobacco products (THS-H) were examined in BEAS-2B human bronchial epithelial cell line.

Materials and Methods: The terrycloth samples were exposed to smoke in a closed polystyrene box and extracted in a complete cell culture medium for 24 hours at 37°C. Following this, the cytotoxicity of THS was assessed by MTT assay. Malondialdehyde (MDA) and intracellular glutathione (GSH) levels were determined in BEAS-2B cell lysate to assess oxidative response. The aryl hydrocarbon receptor (AhR) and interleukine-6 (IL-6) levels were determined via an ELISA kit.

Results: Both types of THS led to dose-dependent cytotoxicity in cells, which was remarkable with THS-C (50%, v/v). Moreover, GSH depletion and MDA increase were remarkable with both THS, particularly with THS-C. AhR activation was also slightly elevated with THS-C, whereas the increase in IL-6 was notable compared to THS-H.

Conclusion: THS exposure might lead to potential health risks particularly for respiratory health and the results support the need for comprehensive regulations and public health initiatives to minimize the harmful effects of THS.

Keywords: Thirdhand smoke, heated-tobacco product, oxidative stress, aryl hydrocarbon receptor, inflammation, interleukine-6.

INTRODUCTION

Smoking is one of the leading causes of preventable death worldwide. Across Organisation for Economic Co-operation and Development (OECD) countries, among smokers aged 15 and over, Turkiye is ranked second with a daily smoking rate of 28% after Indonesia, according to the latest data (1). Due to this relatively high rate of daily smoking, other risk factors may arise not only for active cigarette smokers but also for non-smokers and the environment exposed to the side stream smoke during this process (2). From this point of view, a new toxicological concept, thirdhand smoke (THS), can be described as residual smoke that is remained after the cigarette is extinguished and might be generated from aged secondhand smoke that adheres to dust and surfaces. In addition to its cumulative hazard potential, its re-emission into the air makes the THS a public health concern, particularly for individuals who are suffering from respiratory diseases (3). Even though smoking has been prohibited in most indoor workplaces and public places in Turkiye since 2005, there are a few exceptions where ventilation is allowed for smoking such as care facilities for the elderly people, restricted areas in airports, personal accommodation places, and hotel rooms (4). Therefore,

Corresponding Author: Rengin Reis **E-mail:** rengin.reis@acibadem.edu.tr
Submitted: 25.06.2023 **Revision Requested:** 18.07.2023 **Last Revision Received:** 18.07.2023 **Accepted:** 27.07.2023 **Published Online:** 31.07.2023

indoor smoking might represent a current public health and environmental concern for people who do not actively smoke, despite strengthened bans.

According to the recent literature, the chemical dynamic of THS is quite different from firsthand smoke (FHS) and secondhand cigarette smoke (SHS) due to the delayed and aged exposure to smoke pollutants. In addition, the re-emitted gas phase represents a continuous environmental pollutant reservoir in terms of nicotine, nitrous acid, carcinogenic tobacco-specific nitrosamines (TSNAs), and volatile organic components (VOCs) (5). Unfortunately, physical, and chemical cleaning techniques were shown to be ineffective in removing THS residue adsorbed on surfaces and materials. Also, cleaning methods such as wiping was previously reported to cause particles to become liberated from the deposited surfaces and re-suspended in air, thus increasing the probability of exposure via the inhalation route (6). Exposure via inhalation is a common exposure route for FHS, SHS, and THS; however, due to the surface embedding potential of THS, the oral route is another exposure route for THS, which makes THS a health hazard for toddlers and children who are frequently in contact with surfaces via hand to mouth transfer with a long-lasting effect (7). In addition to the risk to children, people who are suffering from chronic respiratory diseases such as asthma, chronic obstructive pulmonary disease (COPD), and wheezing (8). According to research by Vanker and colleagues (2017), environmental cigarette smoke was suggested as an important public health concern for people, especially children, by increasing the rate of asthma, wheezing, and pulmonary infections. In addition, these health hazards were reported to be at higher rates in low and moderate-income countries in several reports (8,9).

The limited studies on THS exposure showed that it has dose-dependent cytotoxic (10) and genotoxic (11,12) potential on several cell lines and has a delaying effect on wound healing capacity in mice (13). Based on these few findings on the risk of repeated exposure to THS, a toxicological evaluation of the complex mixture of THS and its possible health effects are further needed. In recent years, in addition to the use of conventional cigarettes, "*less harmful*" options to smoke were started to be marketed such as heat-not-burn products or heated tobacco products (HTP). These products aim to serve a smoke containing nicotine to the consumer with a less burning product and carcinogenic intermediates since HTPs are heating the tobacco up to 300°C instead of burning as in traditional cigarettes (14,15). In our previous study, we reported the comparative cytotoxicity of the FHS extracted from HTP and conventional cigarette (3R4F) in human liver epithelial cells, and those novel products dose-dependently might be cytotoxic to the cells via inflammatory and oxidative pathways (16). However, there is no current finding on the residual potential of HTPs and their toxicity potential on the primary target organ, the lungs. Since there is no classified safety level of environmental cigarette smoke exposure, no definitive THS threshold for adverse effects has been recognized. The present study aimed to evaluate the potential cytotoxicity profile of two different types of THSs isolated from conventional cigarette and heated tobacco products preliminarily in human bronchial epithelial cells via oxidative and inflammatory pathways *in vitro*.

MATERIAL AND METHODS

Preparation of THS Extract

THS was extracted from terrycloth fabric exposed to one 3R4F cigarette (THS-C) and heat stick heated via HTP (THS-H) device manually in a polystyrene chamber with a puff volume of 55 mL and 30 seconds of puff cycle as described in the previous smoking simulation (16). After the smoke exposure in the chamber, the terrycloth was extracted in the high glucose Dulbecco`s Modified Eagle Media (DMEM, Gibco, USA) at 37°C for 24 hours. After the extraction process, the extract (100%, v/v) was filtered and stored at -80°C for further studies. The standardization of prepared extract was recorded through before and after weights of filter papers accumulates the total particulate matter (TPM) for each.

Cytotoxicity Assay

The cytotoxicity potential of the two types of THSs was assessed via 3-(4,5-dimethylthiazol-2-yl)−2,5 diphenyltetrazolium bromide (MTT) assay through mitochondrial reductase activity in human bronchial epithelial BEAS-2B cells (ATCC, CRL-9609). For this purpose, cells were cultured with Dulbecco's modified eagle medium (DMEM) supplemented with 100 IU/mL penicillin, 100 µg/mL streptomycin, and 5% (v/v) fetal bovine serum (FBS, Gibco, USA). The cells were seeded in 96-well plates before the day of exposure as $2x10^4$ cells/well and incubated for 24 hours to achieve subconfluency. The following day, THS-C and THS-H were diluted with a complete cell culture medium and applied to the cells between doses at 10-100% (v/v) for 24 hours. After the incubation, the well contents were discarded and 0.5 mg/mL MTT solution was applied to the wells as previously described (17). In addition to the cytotoxicity, cell morphology was also visualized via EVOS M5000 (ThermoScientific, Singapour) at a magnification rate of 10x for 72 hours.

Oxidative Stress

For the assessment of oxidative damage induced by THS-C and THS-H, intracellular glutathione (GSH) and lipid peroxidation intermediate product malondialdehyde (MDA) levels were assessed according to our previous methods (16,17). Briefly, the cells seeded in T-25 flasks were exposed to the 10-25-50% (v/v/v) THS-C and THS-H extracts for 24 hours. The next day, cell pellets were collected and lysed with MagnaLyser (Roche, Switzerland). The collected cell lysates were used for the assessment of total protein content, GSH, and MDA level spectrophotometrically in triplicates (ThermoScientific, VarioskanLUX, Singapour).

Inflammatory Damage

Inflammation is another toxicity response against air pollutants; thus, it was evaluated in BEAS-2B cells via pro-inflammatory cytokine interleukin-6 (IL-6) and aryl hydrocarbon receptor

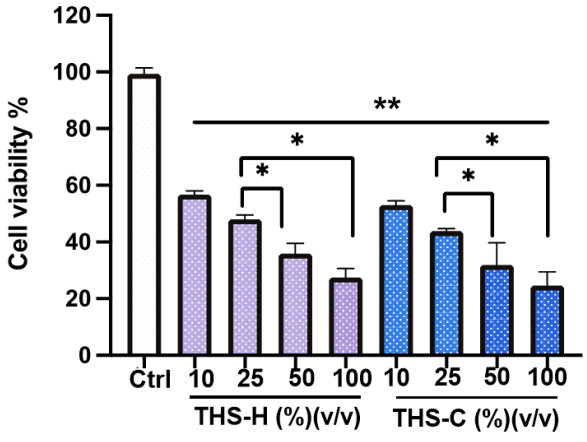

Figure 1. Cytotoxicity profiles of THS-C and THS-H in BEAS-2B cells.
Ctrl: Control; THS-H: Thirdhand smoke of HTP; THS-C: Thirdhand smoke of 3R4F cigarette. Results were expressed as mean± SD. The significant differences between groups and Ctrl were defined as *p<0.05; **p<0.001.

(AhR) levels via enzyme-linked Immuno sorbent assay (ELISA) kit. For this purpose, cell supernatants were assessed with a human IL-6 ELISA kit (Elabscience, E-EL-H6156, USA) and a human AhR ELISA kit (AFG Bioscience, EK700338, USA) at 450 nm spectrophotometrically in duplicates.

Statistical Analyses

Statistical analyses of data were performed with GraphPad Prism 9.0 (La Jolla, California, USA). The results of cell culture studies are presented as the mean ± SD as triplicates. One-way analysis of variance (ANOVA) tests were used for analysis and differences were considered to be significant at p<0.05.

RESULTS

TPM of THS Extracts

The prepared extracts were standardized through collected TPM amounts on the filter papers. Based on our extractions, the TPM of THS-C was recorded as 3.7 ± 0.12 mg whereas for THS-H was 3.1 ± 0.2 mg.

Cytotoxicity

Cytotoxicity of THS-C and THS-H were shown in Figure 1. As seen in Figure 1, both extracts showed dose-dependent cytotoxicity in BEAS-2B cells, which was remarkable with the higher doses of THS-C (50-100%, v/v). Furthermore, all the tested concentrations of THS-C and THS-H were significantly cytotoxic compared to the control group (p<0.001). According to the MTT assay, the inhibiting concentration 50% (IC_{50}) values of extracts were found as 14.8 ± 1.9% for THS-C and 21.7 ± 2.6% for THS-H. The cell morphology of THS-exposed BEAS-2B cells was recorded for 72 hours and shown in Figure 2.

Oxidative Stress

Smoking is a well-known source of free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS) formation, which play a crucial role in oxidative damage in target organs as a notable contributing factor. Hence, we assessed THS induced oxidative damage, which was yielded by a cigarette, and an HTP through intracellular GSH and MDA levels. Based on our findings, 25-50% (v/v) doses of THS-C and THS-H significantly led to a significant depletion in GSH deposits in BEAS-2B cells compared to the control (Figure 3). Moreover, the difference between the highest tested doses was remarkable with THS-C in the GSH assay (p<0.05). On the other hand, lipid peroxidation was significantly higher with THS-C (50%, v/v) compared to the same dose of THS-H (Figure 4) (p<0.001).

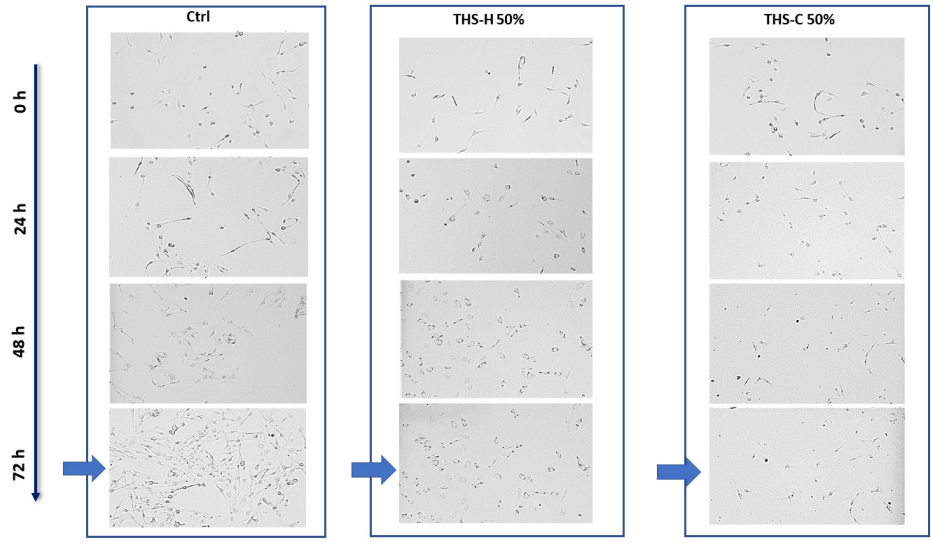

Figure 2. Morphological images of BEAS-2B cells exposed to the THS-C and THS-H for up to 72 hours.

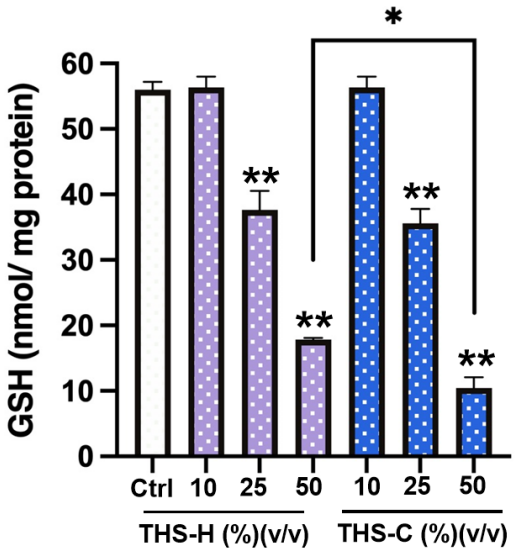

Figure 3. Intracellular GSH level in BEAS-2B cells.
Ctrl: Control; THS-H: Thirdhand smoke of HTP; THS-C: Thirdhand smoke of 3R4F cigarette. Results were expressed as mean± SD. The significant differences between groups and Ctrl were defined with * p< 0.05, **p<0.01

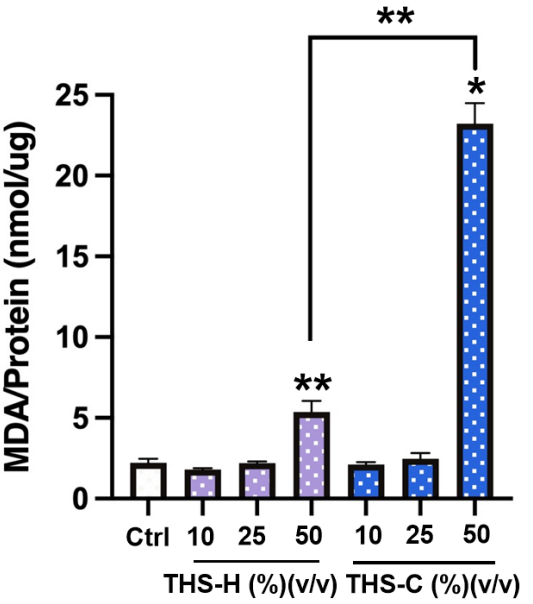

Figure 4. MDA level in BEAS-2B cells.
Ctrl: Control; THS-H: Thirdhand smoke of HTP; THS-C: Thirdhand smoke of 3R4F cigarette. Results were expressed as mean± SD. The significant differences between groups and Ctrl were defined with *p<0.05; **p<0.001

Inflammatory Response

IL-6 Levels

Inflammatory response through pro-inflammatory IL-6 level was found to be elevated with THS-C and THS-H exposure dose-dependently as shown in Figure 5. Based on the ELISA results, 50% and 100% (v/v) doses of the extracts exhibited a significant increase in the inflammatory response, which was the highest with THS-C. In addition, the difference in the IL-6 release was statistically significant between the highest tested doses of THSs (p<0.05).

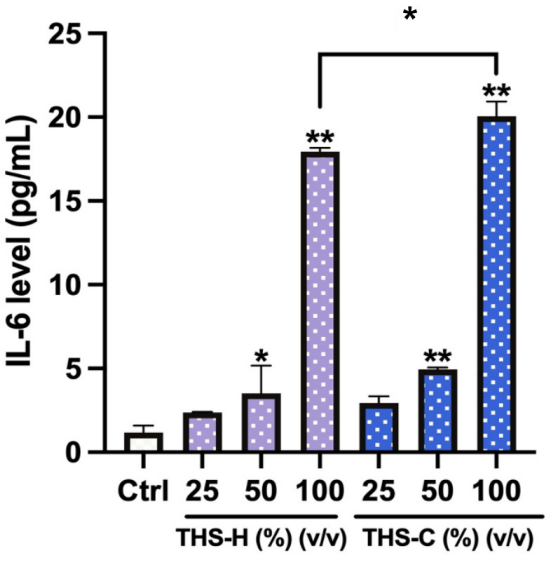

Figure 5. IL-6 level in BEAS-2B cells.
Ctrl: Control; THS-H: Thirdhand smoke of HTP; THS-C: Thirdhand smoke of 3R4F cigarette. Results were expressed as mean± SD. The significant differences between groups and Ctrl were defined with *p <0.05, **p<0.001.

AhR Levels

Another important inflammatory marker, AhR, which has specific ligands such as tobacco smoke cigarette smoke carcinogen benzo(α)pyrene, was evaluated in BEAS-2B cells via ELISA kit. According to the present findings, cytoplasmic AhR level was higher with THS-H (100%, v/v) exposure compared to the same dose of THS-C (Figure 6). However, the difference between the two groups was insignificant.

DISCUSSION

In the present study, we focused on the potential cytotoxicity, oxidative stress, and inflammatory response caused by THS derived from conventional cigarettes and heated tobacco products. The findings indicate that both THS-C and THS-H showed dose-dependent cytotoxicity in BEAS-2B cells, where the higher doses of THS-C exhibited more pronounced cytotoxic effects compared to THS-H based on their IC_{50}. Moreover, the cell morphology analysis further supported the cytotoxic effects of THS on the cells. Similar to our findings, Bahl and colleagues reported a dose-dependent cigarette-derived-THS cytotoxicity in human dermal fibroblasts (hDF)

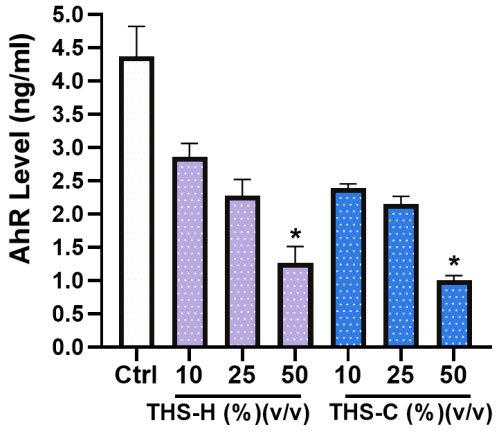

Figure 6. Cytoplasmic AhR level in BEAS-2B cells.
Ctrl: Control; THS-H: Thirdhand smoke of HTP; THS-C: Thirdhand smoke of 3R4F cigarette. Results were expressed as mean± SD. The significant differences between groups and Ctrl were defined with * p<0.001.

and human palatal mesenchyme cells (hPM) (10). In another study, indoor surface-derived THS was also found dose-dependently cytotoxic to mouse neural stem cells (mNSC), which represented a model for the neonatal brain (18). However, there are no records in the literature on the HTP-derived THS and potential cytotoxicity profile. Therefore, this is the first comparative report that identifies the cytotoxicity profile of HTP-derived THS *in vitro*. Oxidative stress is another important pathway involved in chronic pulmonary diseases and tobacco-induced lung deficits (19–21), and its assessment revealed that both THS-C and THS-H led to a depletion in intracellular GSH deposits in BEAS-2B cells. THS-C induced higher levels of lipid peroxidation compared to THS-H exposure, indicating its stronger potential for oxidative damage based on our findings. The other important pathway involved in respiratory diseases is inflammation, which was augmented with smoking as well, and was evaluated with pro-inflammatory IL-6 and tobacco-specific carcinogen ligand-induced-AhR level (22-25). According to the results, THS-C and THS-H exposure exhibited a dose-dependent increase in IL-6 levels, with THS-C showing a higher inflammatory response. However, there was no significant difference in cytoplasmic AhR levels between THS-C and THS-H. Previously, we reported a slight increase in HepG2 cells exposed to the FHS and SHS extracts of both conventional cigarettes and HTP, which was higher with cigarettes (16). Similarly, THS derived from a cigarette led to a higher AhR activation with a lower cytoplasmic AhR level in the current study. Based on these findings, it might be suggested that both types of THS demonstrated dose-dependent cytotoxicity, with THS-C exhibiting stronger effects. The oxidative stress assessment indicated a depletion of intracellular GSH deposits and higher lipid peroxidation with THS-C. Furthermore, THS-C induces a stronger inflammatory response through IL-6 release in BEAS-2B cells.

CONCLUSION

These findings emphasize the potential health risks associated with THS exposure, particularly in terms of respiratory health. The study contributes to the understanding of the toxicological profile of THS and highlights the importance of further research to assess the long-term effects of THS exposure on human health. The results support the need for comprehensive regulations and public health initiatives to minimize THS exposure and protect individuals, especially those with respiratory conditions, from the harmful effects of THS.

Acknowledgments: This study was presented as an oral presentation at the 11th International Turkish Society of Toxicology Congress, Antalya, Turkiye (2-5 November 2022).

Ethics Committee Approval: Ethics committee approval is not required for cell culture studies in the article.

Authors' Contributions: Conception/Design of Study- K.K., R.R.; Data Acquisition – K.K., R.R.; Data Analysis/Interpretation – K.K., R.R.; Drafting Manuscript– R.R.; Critical Revision of Manuscript- R.R.; Final Approval and Accountability– K.K., R.R.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

REFERENCES

1. OECD. OECD-Daily smoking rates (cited in June, 2023). Available from URL: https://data.oecd.org/healthrisk/daily-smokers.htm
2. Pagani LS. Environmental tobacco smoke exposure and brain development: The case of attention deficit/hyperactivity disorder. Neurosci Biobehav Rev 2014; 44: 195–205.
3. Matt GE, Quintana PJE, Hoh E, Dodder NG, Mahabee-Gittens EM, Padilla S, et al. Tobacco smoke is a likely source of lead and cadmium in settled house dust. J Trace Elem Med Biol 2021; 63: 126-36.
4. Tobacco Control Law. WHO Framework Convention on Tobacco Control-2022 (cited in June, 2023). Available from URL: https://www.tobaccocontrollaws.org/legislation/turkey#:~:text=Smoking is prohibited in most,%2C prisons%2C and hotel rooms.
5. Moon SY, Kim T, Kim YJ, Kim Y, Kim SY, Kang D. Public facility utility and third-hand smoking exposure without first and second-hand smoking according to urinary cotinine level. Int J Environ Res Public Health 2019; 16(5): 855.
6. Kuo HW, Rees VW. Third-hand smoke (THS): What is it and what should we do about it? J Formos Med Assoc 2019; 118: 1478–79.
7. Arguder E. Third-hand smoke exposure and results. Eurasian J Pulmonol 2019; 21: 81-6
8. Vanker A, Gie RP, Zar HJ. The association between environmental tobacco smoke exposure and childhood respiratory disease: a review. Expert Rev Respir Med 2017; 11: 661–73.
9. Zafeiridou M, Hopkinson NS, Voulvoulis N. Cigarette smoking: an assessment of tobacco's global environmental footprint across its entire supply chain. Environ Sci Technol 2018; 52(15): 8087–94.
10. Bahl V, Shim HJ, Jacob P, Dias K, Schick SF, Talbot P. Thirdhand smoke: chemical dynamics, cytotoxicity, and genotoxicity in outdoor and indoor environments. Toxicol in Vitro 2016; 32: 220–31.

11. Hang B, Wang P, Zhao Y, Chang H, Mao JH, Snijders AM. Thirdhand smoke: Genotoxicity and carcinogenic potential. Chronic Dis Transl Med 2020; 6(1): 27–34.
12. Hang B, Sarker AH, Havel C, Saha S, Hazra TK, Schick S, et al. Thirdhand smoke causes DNA damage in human cells. Mutagenesis 2013; 28(4): 381–91.
13. Martins-Green M, Adhami N, Frankos M, Valdez M, Goodwin B, Lyubovitsky J, et al. Cigarette smoke toxins deposited on surfaces: Implications for human health. PLoS One 2014; 9(1): 1–12.
14. Glantz S. Heated tobacco products: The example of IQOS. Tob Control 2018; 27: 3–6.
15. Lee CM. The impact of heated tobacco products on smoking cessation, tobacco use, and tobacco sales in South Korea. Korean J Fam Med 2020; 41(5): 273–81.
16. Reis R, Kolci K, Bahcivan İ, Coskun GP, Sipahi H. Alpha-lipoic acid modulates the oxidative and inflammatory responses induced by traditional and novel tobacco products in human liver epithelial cells. Chem Biodivers 2023; 20(3): e202200928.
17. Reis R, Orak D, Yilmaz D, Cimen H, Sipahi H. Modulation of cigarette smoke extract-induced human bronchial epithelial damage by eucalyptol and curcumin. Hum Exp Toxicol 2021; 40(9): 1445–62.
18. Bahl V, Weng NJH, Schick SF, Sleiman M, Whitehead J, Ibarra A, et al. Cytotoxicity of thirdhand smoke and identification of acrolein as a volatile thirdhand smoke chemical that inhibits cell proliferation. Toxicol Sci 2016; 150(1): 234–46.
19. Hosseini MJ, Naserzadeh P, Salimi A, Pourahmad J. Toxicity of cigarette smoke on isolated lung, heart, and brain mitochondria: induction of oxidative stress and cytochrome c release. Toxicol Environ Chem 2013; 95(9): 1624–37.
20. Schweitzer KS, Hatoum H, Brown MB, Gupta M, Justice MJ, Beteck B, et al. Mechanisms of lung endothelial barrier disruption induced by cigarette smoke: role of oxidative stress and ceramides. Am J Physiol Lung Cell Mol Physiol 2011; 301(6): 836–46.
21. Kluchová Z, Petrásová D, Joppa P, Dorková Z, Tkácová R. The association between oxidative stress and obstructive lung impairment in patients with COPD. Physiol Res 2007; 56(1): 51–6.
22. Lugade AA, Bogner PN, Thatcher TH, Sime PJ, Phipps RP, Thanavala Y. Cigarette smoke exposure exacerbates lung inflammation and compromises immunity to bacterial Infection. J Immunol 2014; 192(11): 5226–35.
23. Moghaddam SJ, Barta P, Mirabolfathinejad SG, Ammar-Aouchiche Z, Garza NT, Vo TT, et al. Curcumin inhibits COPD-like airway inflammation and lung cancer progression in mice. Carcinogenesis 2009; 11: 1949–56.
24. Boskabady MH, Gholami Mahtaj L. Lung inflammation changes and oxidative stress induced by cigarette smoke exposure in guinea pigs affected by Zataria multiflora and its constituent, carvacrol. BMC Complement Altern Med 2015; 15: 39.
25. Radan M, Dianat M, Badavi M, Mard SA, Bayati V, Ahmadizadeh M. The association of cigarette smoke exposure with lung cellular toxicity and oxidative stress: the protective role of crocin. Inflammation 2020; 43(1): 135–45.