Direct Effects of Waterpipe Smoke Extract on Aortic Endothelial Cells: An In Vitro Study

Nur Elena ZAABA¹*, Priya YUVARAJU²*, Sumaya BEEGAM¹, Ozaz ELZAKI¹, Kholoud ARAFAT², Samir ATTOUB², Abderrahim NEMMAR¹

* These authors contributed equally to this work.

¹Department of Physiology, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates, ²Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates

Received May 17, 2024 Accepted October 3, 2024

Summary

Waterpipe smoking (WPS) has adverse health effects that include endothelial dysfunction with mechanisms involving oxidative stress and inflammation. Nonetheless, there is a scarcity of data on the direct impact of WPS on endothelial function. In this study, we assessed the in vitro effects of waterpipe smoke extract (WPSE) on aortic endothelial cell lines, namely the TeloHAEC. The WPSE markedly caused concentration- and timedependent decreases in cellular viability. When compared with the control, at a concentration of 20 % and an incubation period of 48 h, the WPSE significantly increased the levels of lactate dehydrogenase, and markers of oxidative stress including thiobarbituric acid reactive substances, superoxide dismutase, catalase, and reduced glutathione. Moreover, the concentrations of proinflammatory cytokine (tumor necrosis factor a), and adhesion molecules (E-selectin and intercellular adhesion molecule-1) were also significantly augmented. Likewise, WPSE triggered mitochondrial dysfunction, DNA oxidative damage, as well as apoptosis in TeloHAEC cells. Similarly, cells cultured with WPSE have shown increased expression of phosphorylated nuclear factor-kB and hypoxia-inducible factor 1-a (HIF-1a). In conclusion, our study showed that WPSE triggers endothelial inflammation, oxidative stress, DNA damage, mitochondrial dysfunction, and apoptosis via mechanisms involving the activation of nuclear factor-kB and HIF-1a.

Key words

Waterpipe smoking • Aortic endothelial cells • Inflammation • Oxidative Stress

Corresponding author

A. Nemmar, Department of Physiology, College of Medicine and Health Sciences, United Arab Emirates University, P.O. Box 15551, Al Ain, United Arab Emirates. E-mail: anemmar@uaeu.ac.ae

Introduction

Waterpipe smoking (WPS), also known as hookah or shisha is a growing phenomenon worldwide, particularly among young adults and women, and has become a growing public health issue [1]. Several factors are attributed to WPS's popularity, including the use of fruit-flavored tobacco, which produces pleasant fruity aromas and tastes [2]. Moreover, the rise in popularity of WPS can also be ascribed to the misconception that the fruity and cool smoke inhaled is less hazardous and less addictive compared to cigarette smoking (CS) [3,4]. However, there is now a substantial body of evidence to debunk these misconceptions and link WPS to adverse health effects, with a particular emphasis on its effect on endothelial and cardiovascular health [5].

Waterpipe (WP) smoke is a complex concoction of various toxic elements, comprising carbon monoxide, nicotine, heavy metals, polycyclic and heterocyclic aromatic hydrocarbons, volatile organic compounds, and particulate matter [1,5]. These toxic compounds are the products of the combustion of heated tobacco and from the burning of the charcoal used to heat the tobacco. Previous epidemiological and clinical studies have shown that contrary to popular belief WPS is in fact more

PHYSIOLOGICAL RESEARCH • ISSN 1802-9973 An open access article under the CC BY 4.0 license <u>https://creativecommons.org/licenses/by/4.0/</u> © 2025 by the authors. Published by the Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic. E-mail: physres@fgu.cas.cz, www.biomed.cas.cz/physiolres

harmful than CS [6]. It has been reported that one session of WPS could produce up to 6.5 times the amount of carbon monoxide, 1.7 times more nicotine, and 46 times more tar relative to a session of a single cigarette [6]. This is probably due to the longer smoking session and deeper inhalation compared to CS [6].

Human telomerase reverse transcriptaseimmortalized aortic endothelial cells (TeloHAEC) are cells that demonstrate the characteristics of endothelial cells in the aorta and heart. The endothelial cells play various roles in cardiovascular homeostasis, as they regulate the vascular tone, thrombosis, angiogenesis, and inflammation [7]. Moreover, the endothelial cells are the second most exposed cells after the alveolar cells, and are known to be vulnerable to the deleterious effects caused by the products of tobacco combustion [8]. In response to cardiovascular risk factors, including WPS, the endothelial cells release large amounts of vasoactive substances and inflammatory mediators [8]. This could potentially alter normal endothelial functions and lead to vascular endothelial dysfunction, which precedes the development of atherosclerosis and coronary heart disease [8].

The adverse in vivo effects of WPS on endothelial and cardiovascular health have been previously described [9-11]. We have recently demonstrated that WPS inhalation triggers lung injury and endothelial inflammation, oxidative stress, and apoptosis, which were associated with nuclear factor-kB activation and SIRT1 down-regulation [10]. These findings led us to speculate whether these effects were due to the spill-over of inflammatory mediators and pro-oxidants from the injured lungs into the circulatory system, or whether they were a result of direct contact between WP smoke toxicants crossing the alveolarcapillary barrier and interacting directly with vascular endothelium. Therefore, in order to explore this aspect, it is essential that we shift our focus from in vivo mainstream WPS to study the effect of waterpipe smoke extract (WPSE) on endothelial cells, which are the focal cells of the vascular system.

Consequently, the objective of our study is to evaluate the possible direct impact of WPSE on aortic endothelial cells in vitro and the underlying mechanisms involved by assessing various relevant parameters including markers of oxidative stress, inflammation, mitochondrial dysfunction, DNA oxidative damage, and apoptosis.

Material and Methods

Setup and preparation of waterpipe smoke extract

Figure 1 depicts a modified version of a previously described set up pertaining with the preparation of WPSE [2]. Ten grams of apple-flavored tobacco purchased from Al Fakher Tobacco Trading (Ajman, UAE) were placed in a shisha bowl, covered with pierced aluminum foil, and topped with red hot charcoal to create waterpipe smoke extract [2]. The smoke was channeled through distilled water in a glass vase. Tobacco smoke from the waterpipe was collected into 10 ml of serum-free endothelial basal medium (EBMTM)-2 media from Lonza (Walkersville, MD, USA) using vacuum suction in a 50 ml centrifuge tube at a flow rate of 35 ml/min. The optical density of the WPSE obtained was systematically measured each time before the exposure of WPSE to TeloHAEC cells in order to ensure that the nicotine content was consistent with that of a regular WPS session. A delta OD of 3±0.2 was found to be equivalent to 100 % WPSE when measured at 320 and 540 nm [12,13]. The resulting WPSE was used further to study the cell viability on TeloHAEC cells.

> Fig. 1. The schematic diagram represents the preparation of waterpipe smoke extract (WPSE) from flavored tobacco smoke in the shisha The smoke produced by set-up. burning flavored tobacco over charcoal (A). The distilled water in the glass vase functions as a conduit for the tobacco smoke (B). The tobacco smoke movement into a 10 ml serumfree EBM[™]-2 Clonetics® media is shown by an arrow in the pipeline (C). The smoke was drawn at a constant rate of 35 ml/min using a flow meter (D). Negative pressure was regulated by vacuum suction (E).





Cell culture and reagents

The TeloHAEC (hTERT-immortalized human aortic endothelial cells) were maintained in EMB-2 medium kit (CC-3162, Lonza, Walkersville, MD, USA) at 37 °C in a 5 % CO₂ atmosphere. The WPSE was diluted in full medium until it reached the desired concentration of 20 %.

Cell viability

TeloHAEC cells were seeded into 96-well plates at a density of 9000 cells/well (n=4 independent experiments). After 24 h [14], cells were incubated with WPSE at 1, 5, 10 and 20 % in duplicate for 1, 6, 24 and 48 h while control cells were incubated in media without WPSE. A CellTiter-Glo® Luminescent Cell Viability assay from Promega Corporation (Madison, WI, USA) was used at the indicated time points to determine the effects of WPSE on cellular viability by quantifying the adenosine triphosphate (ATP) that is proportional to the number of metabolically active cells. The luminescence was evaluated with a GloMax® Luminometer by Promega Corporation (Madison, WI, USA). By comparing WPSE-treated cells to control cells, where viability was arbitrarily set at 100 %, we were able to express cellular viability as a percentage (%).

Biochemical markers

TeloHAEC cells were seeded into 12-well plates at a density of 80000 cells/well (n=4 independent experiments). After 24 h, the cells were incubated with WPSE at a concentration of 20 % in 1 ml of culture media per well for 48 h while control cells were incubated in 1 ml of the same media without WPSE. Supernatants of the culture media were then collected and analyzed for several biochemical markers.

Lactate dehydrogenase (LDH)

The release of LDH was evaluated as a surrogate marker of cell damage using a modified assay as previously described [15]. Briefly, in a 96 well plate, 50 μ l of assay reagent (made by combining 1-methoxyphenazine methosulfate, iodonitrotetrazolium chloride, and β -nicotinamide adenine dinucleotide sodium salt a few minutes before starting the assay) was added to a 50 μ l cell culture media supernatant. After a quick shake on an orbital shaker, the plate was left to incubate for 60 min at room temperature in the dark. Finally, 50 μ l of 1 M acetic acid was added to each well to terminate the reaction and stabilize it. A plate reader was used to measure the absorbance at 490 nm.

Thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), catalase and reduced glutathione (GSH)

Cayman Chemicals kits (Ann Arbor, MI, USA) were used to measure TBARS, SOD and catalase activities. Whereas the concentration of GSH was measured using a kit purchased from Sigma Aldrich Co. (St. Louis, MO, USA) [16].

Tumor necrosis factor α (*TNF* α), *E-selectin and intercellular adhesion molecule-1 (ICAM-1)*

The concentrations of TNF α , E-selectin and ICAM-1 were assessed using ELISA kits purchased from R&D Systems (Minneapolis, MN, USA).

Mitochondrial membrane potential analysis with JC-1 probe

A kit from Cayman Chemicals (Ann Arbor, MI, USA) was used to perform the JC-1 mitochondrial membrane potential assay.

DNA oxidative damage

The level of 8-hydroxy-2-deoxyguanosine (8-OHdG) was measured using an ELISA kit from Cayman Chemicals (Ann Arbor, MI, USA).

Cytochrome C and cleaved caspase 3

Cytochrome C and cleaved caspase 3 were quantified using ELISA kits from R&D Systems (Minneapolis, MN, USA).

Phosphorylated nuclear factor κB (Phospho-NF- κB) and Hypoxia-inducible factor 1- α (HIF-1 α)

The concentration of phospho-NF- κ B was measured using a kit from Cell Signalling Technology (Danvers, MA, USA), whereas the concentration of HIF-1 α was determined by using an ELISA kit from R&D Systems (Minneapolis, MN, USA).

Results

Cell viability

The effect of the increasing concentration of WPSE was investigated on the TeloHAEC cell lines. As shown in Figure 2, the WPSE significantly reduced the viability of TeloHAEC in concentration- and time-dependent manner. The half-maximal inhibitory concentration (IC_{50}) of WPSE at 48 h was approximately at 20 % of WPSE, hence cells incubated with this concentration and at this duration was selected to represent the WPSE group for the subsequent analyses.

LDH

The cytotoxic effects of WPSE on TeloHAEC cell lines were evaluated using the LDH assay. Figure 3 shows that when compared with the control group, cells incubated in 20 % WPSE displayed a significant increase in the level of LDH (p<0.05).

TBARS, catalase, SOD, GSH

In order to evaluate the oxidative stress of cells incubated with WPSE versus the control, we have quantified the marker of lipid peroxidation, TBARS. As depicted in Figure 4, the level of TBARS was significantly elevated (p=0.0001) in cells exposed to 20 % WPSE, compared with the control group. Likewise, a significant increase in catalase (p<0.0001) and SOD (p=0.001) activities, and GSH (p<0.001) concentration were observed in WPSE-treated cells compared with the control group.

TNF a, *E*-selectin, *ICAM-1*, *mitochondrial membrane* potential analysis with *JC-1* probe and *DNA* oxidative damage

Figure 5A-C shows the concentrations of the TNF α , as well as the adhesion molecules E-selectin and ICAM-1 in TeloHAEC cells incubated with WPSE compared with the control group. The concentrations of TNF α (*p*<0.05), E-selectin (*p*<0.01) and ICAM-1 (*p*<0.05) were significantly increased in WPSE group compared with the control.

Figure 5D depicts that WPSE group has significantly lower JC-1 fluorescence ratio (p < 0.0001) compared with the control group.

When compared with the control group, cells incubated with 20 % WPSE released significantly higher level of the oxidized derivative of deoxyguanosine 8-OH-dG (p<0.0001, Fig. 5E), a major product of DNA oxidation.

Cytochrome C, cleaved caspase 3, phospho-NF- κB and HIF-1 α

Figure 6A-B shows that there was a significant increase in the levels of cytochrome C (p<0.0001) and cleaved caspase 3 (p=0.0001) in WPSE group compared with the control.

Figure 6C shows that cells incubated with 20 % WPSE had a significantly higher level of phospho-NF- κ B compared with the control (*p*<0.0001).

Figure 6D depicts that the concentration of HIF-1 α was remarkably increased in WPSE-exposed cells, compared with the control group (*p*<0.0001).



Fig. 2. Cell viability assay for TeloHAEC cells after 1, 6, 24 and 48 h of incubation with waterpipe smoke extract (WPSE) at different concentrations (0, 1, 5, 10 and 20 %). Cellular viability is significantly reduced after 48 h incubation with 20 % WPSE. Mean ± SEM (at least 3 independent experiments). Statistical analysis by one-way analysis of variance followed by Holm-Sidak's test. ^{ΔΔ} *p*<0.01 group incubated with 20 % WPSE for 48 h compared with the group incubated with 0 % WPSE for 1 h and ^{ΔΔΔΔ} *p*<0.001 group incubated with 0 % WPSE for 1 h. ** *p*<0.01 group incubated with 0 % WPSE for 1 h. ** *p*<0.01 group incubated with 0 % WPSE for 48 h compared with the group incubated with 0 % WPSE for 48 h compared with the group incubated with 0 % WPSE for 48 h and **** *p*<0.0001 group incubated with 20 % WPSE for 48 h and **** *p*<0.0001 group incubated with 20 % WPSE for 48 h compared with the group treated with 0 % WPSE for 48 h and **** *p*<0.0001 group incubated with 0 % WPSE for 48 h compared with the group treated with 0 % WPSE for 48 h compared with the group treated with 0 % WPSE for 48 h and **** *p*<0.0001 group incubated with 0 % WPSE for 48 h compared with the group treated with 0 % WPSE for 48 h compared with the group treated with 0 % WPSE for 48 h compared with the group treated with 0 % WPSE for 48 h compared with the group treated with 0 % WPSE for 48 h compared with the group treated with 0 % WPSE for 48 h compared with the group treated with 0 % WPSE for 48 h.



Fig. 3. Lactate dehydrogenase (LDH) released in the culture media of TeloHAEC cells incubated with 20 % waterpipe smoke extract (WPSE) and in media with 0 % WPSE for 48 h. Mean \pm SEM (n=4 independent experiments). Statistical analysis by one-way analysis of variance followed by Holm-Sidak's test.

Discussion

The present study has provided experimental evidence of the adverse effects of the *in vitro* exposure to WPSE on the aortic endothelial cells involving mechanisms including oxidative stress, inflammation, DNA damage and apoptosis, all of which are crucial in the development and the progression of endothelial dysfunction.



Fig. 4. Thiobarbituric acid reactive substances (TBARS, **A**), catalase (**B**), superoxide dismutase (SOD, **C**) and reduced glutathione (GSH, **D**) released in the culture media of TeloHAEC cells incubated with 20 % waterpipe smoke extract (WPSE) and in media with 0 % WPSE for 48 h. Mean ± SEM (n=4 independent experiments). Statistical analysis by one-way analysis of variance followed by Holm-Sidak's test.



Fig. 5. Tumor necrosis factor a (TNF a, **A**), E-selectin (**B**), intercellular adhesion molecule-1 (ICAM-1, **C**), JC-1 mitochondrial membrane potential (**D**) and 8-hydroxy-2-deoxyguanosine (8-OH-dG, **E**) released in the culture media of TeloHAEC cells incubated with 20 % waterpipe smoke extract (WPSE) and in media with 0 % WPSE for 48 h. Mean \pm SEM (n=4 independent experiments). Statistical analysis by one-way analysis of variance followed by Holm-Sidak's test.

Both epidemiological and experimental studies have shown that WPS is associated with the deterioration of the vascular endothelium. This significantly increases the risk of endothelial dysfunction which precedes various clinical manifestation of coronary artery diseases including atherosclerosis, thrombosis, and sudden cardiac death [10]. The adverse vascular endothelial effects associated with WPS can be ascribed to the intricate interplay between oxidative stress and inflammation [17]. These mechanisms alter the normal endothelial functions, lead to several adverse effects and such as vasoconstriction, thrombosis and apoptosis which are the precursors of cardiovascular diseases [18]. It has been hypothesized that inhaled toxicants induces oxidative stress and inflammation either by direct contact with the endothelium, or indirectly where the toxicants cause oxidative stress and inflammation locally in the lungs which can lead to the release of proinflammatory cytokines



Fig. 6. Cytochrome C (**A**), cleaved caspase 3 (**B**), phosphorylated nuclear factor- κ B (phospho-NF- κ B, **C**) and hypoxia-inducible factor 1-a (HIF-1a, **D**) released in the culture media of TeloHAEC cells incubated with 20 % waterpipe smoke extract (WPSE) and in media with 0 % WPSE for 48 h. Mean ± SEM (n=4 independent experiments). Statistical analysis by one-way analysis of variance followed by Holm-Sidak's test.

and pro-oxidants which can cross the alveolar capillary barrier and reach the systemic circulation [19-21].

We have previously elucidated the adverse in vivo endothelial effects of one month exposure to WPS in mice, where WPS induced lung injury and increased the aortic concentrations of proinflammatory cytokines as well as markers of oxidative stress [10]. Moreover, the same observation was found in the heart of mice, along with an increase in prothrombic events [22]. This information led us to speculate whether these effects were due to the spill-over of pro-oxidants and inflammatory mediators from the lungs to the circulatory system following WPS inhalation, or perhaps were the results of a direct contact between the WP smoke toxicants with the vascular endothelium. The present study was designed to provide essential information on the direct impact of WPSE on vascular endothelium particularly on the aortic endothelial cells.

In this study, TeloHAEC endothelial cells from human aorta were incubated in media containing WPSE at the concentration of 20 % for the duration of 48 h. Prior to that, we have carried out the cell viability assay to determine the half-maximal inhibitory concentration of the WPSE, and found that at 20 % of concentration and 48 h of incubation period the extract has shown maximum efficiency by inhibiting the biological processes of half of the population of cells, hence, selected as the most suitable concentration and time point [23]. We subsequently assessed the level of LDH, a biomarker of cytotoxicity which could indicate endothelial cell damage and found to be elevated in various diseases, including coronary artery disease [24]. In fact the measure of LDH in cell culture medium could provide accurate measurement of cell viability [15]. Presently, we found that the concentration of LDH was significantly higher in cells treated with WPSE compared to the control.

In light of the latter observations, we wanted to investigate the involvement of oxidative stress in the present endothelial cytotoxicity by assessing several markers of oxidative stress including TBARS, one of the many reactive aldehydes produced as a result of lipid peroxidation [25-28]. We found that the concentration of TBARS was significantly elevated in the WPSE group compared with the control group, an observation that was confirmed by a previous study that delineates the effect of cigarette smoke extract (CSE) on human bronchial epithelial cells [29]. Similarly, the activities of SOD and catalase and the concentration of GSH were significantly augmented in cells exposed to WPSE compared with the control group. The consistent increase in all antioxidant parameters is possibly due to the compensatory mechanism to counterbalance the oxidative stress environment, during which the cells produce more antioxidants to neutralize the reactive oxygen species (ROS) induced by the toxicants in WP smoke [25]. These observations suggest that the vascular oxidative stress caused by WP smoke is probably due to the ability of WP smoke toxicants to directly induce oxidative stress in the endothelium. Likewise, the concentration of the proinflammatory cytokine TNF α was significantly higher in cells exposed to WPSE compared with the control group, suggesting that in addition to oxidative stress, inflammation could also be directly induced by WP smoke toxicants. These findings corroborate with previous clinical and experimental studies where WPS has been reported to cause systemic and vascular oxidative stress and inflammation [5,8,10].

inflammation, During the release of proinflammatory cytokines in the inflamed site induces the expression of endothelial-cell specific adhesion proteins, particularly E-selectin and ICAM-1 [30]. These important markers of endothelial disorder are responsible in recruiting and transporting leukocyte to the site of inflammation [31]. Here. we found that the concentrations of E-selectin and ICAM-1 were significantly elevated in the WPSE group, which is consistent with our previous in vivo findings in the aortic tissue and plasma of mice exposed to WPS [10,11].

Several studies have reported that the toxicant in tobacco smoke could directly alter the mitochondrial DNA and inhibit mitochondrial proteins in cardiac and vascular tissues in mice and rabbits [32]. This subsequently leads to the reduction of the mitochondrial membrane potential [33]. Our findings are in agreement with these reports, as we found a significant reduction in mitochondrial membrane potential in the aortic endothelial cells exposed to WPSE compared with the control. The mitochondrial membrane potential plays a pivotal role in oxidative phosphorylation, the latter forms transmembrane potential of hydrogen ion, an essential component for ATP production [34]. Subsequently, the reduction of the mitochondrial membrane potential leads to the reduction of ATP produced and the increase of free radical production, which could exacerbate the oxidative stress and inflammation, and consequently cause damage to cellular components, including DNA [25,35]. In the present study, we have evaluated the level of 8-OH-dG, one of the most predominant oxidative lesions caused by ROS, that affect both nuclear and mitochondrial DNA [36], and found that the concentration of this marker was significantly elevated in WPSE group compared with the control group. The presence of DNA damage could trigger both cell arrest and DNA repair mechanisms. However, some of the oxidative DNA damage might not be properly repaired due to the severity of the damage and

subsequently could lead to apoptosis, a programmed cell death [37].

It is well established that the mitochondrialinduced apoptosis occurs intrinsically and initiated upon the release of cytochrome C into the cytoplasm as a result of cellular oxidative stress or damage [38]. Cytochrome C then, recruits and activates caspase 9, which in turn partakes in the cleavage and the activation of caspase 3, an enzyme that is critical in the execution of apoptosis [38]. In addition to that, the extrinsic pathway led by TNF α could initiate apoptosis as well [39]. Presently, we found that WPSE group expressed significantly higher levels of cytochrome C and cleaved caspase 3, compared with the control group. These observations are in line with our previous in vivo observations which showed that WPS induces oxidative stress leading to the elevation of cytochrome C and cleaved caspase 3 activity in heart and aortic tissues, suggesting mitochondrial damage and the initiation of apoptosis [10]. The elevated concentrations of TNF α and cytochrome C in this study suggests that it is possible that apoptosis was initiated via both intrinsic and extrinsic pathways, which converge on caspase 3 activation and subsequently led to the increase of its level [40]. These findings corroborate the previously reported data, including our own, where we have elucidated the apoptotic effects of WPS in the aorta of mice [10]. Moreover, when exposed to CSE, overexpression of caspase 3 was observed in human umbilical venous endothelial cells [41]. While another study showed that CSE caused DNA-strand breaks and induced the activation of the pro-apoptotic marker P53, which resulted in apoptosis, as well as mitochondrial membrane depolarization [42].

To gain a deeper insight into the mechanisms responsible for the adverse effects of WPS, we have evaluated the levels of phospho-NF- κ B. Here, we found that phospho-NF- κ B was markedly elevated in WPSE group compared with the control group, which concurred with previous reports [10,43]. In human epithelial cells, it has been reported that CSE induced the activation of the NF- κ B [43]. Moreover, we have previously reported that in mice, WPS caused significant elevation in the level of phospho-NF- κ B in the aorta [10]. This suggests that the initiation of inflammation might be, at least in part, due to the activation of the NF- κ B.

Furthermore, we have evaluated the concentration of HIF-1 α . The latter is an important marker for hypoxia induced by oxygen deprivation which can be triggered by various external factors including

cigarette smoking [44]. During hypoxia, the expression of HIF-1 α is heavily regulated by the NF- κ B pathway [45]. Previous study has shown the interdependent relationship between HIF-1a and NF-kB, using luciferase under the control of NF-kB-expressing transgenic mice to demonstrate that hypoxia activates NF-KB in the heart and lungs at normobaric atmosphere [45]. Moreover, the same study reported the suppression of hypoxia-induced HIF-1 α in cells in which the canonical NF- κ B pathway has been inhibited [45]. These findings suggest that HIF-1 α plays an important role in inflammation. Additionally, several studies have indicated that the mitochondrial ROS alone can activate HIF-1a, while others suggest that in addition to NF-kB, ROS and the MAPK/AKT signalling could modulate the HIF-1a activation [44]. In the present study, the concentration of HIF-1a was significantly elevated in WPSE group compared with the control. The aforementioned observation coincides with a previous study on CSE on alveolar and bronchial epithelium-derived cells, where the level of HIF-1a increased in a ROS-dependent manner which subsequently led to inflammation and apoptosis [44].

In conclusion, the aortic endothelial cells exposed to 20 % WPSE for 48 h exhibited oxidative stress, inflammation, mitochondrial dysfunction which subsequently led to DNA damage and apoptosis. These findings suggest that the toxicants in the WPSE could potentially cross the alveolar-capillary barrier and directly cause oxidative stress and inflammation in the endothelial cells. However, further work is warranted in order to determine which toxicants are specifically responsible for the adverse effects, and the mechanisms involved in this action.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This research was funded by Zayed Center for Health Sciences (grant number 12R166), College of Medicine and Health Sciences (grant number 12M112) and UPAR (grant number 12M098) of the United Arab Emirates University.

References

- Taati B, Arazi H, Suzuki K. Oxidative Stress and Inflammation Induced by Waterpipe Tobacco Smoking Despite Possible Protective Effects of Exercise Training: A Review of the Literature. Antioxidants (Basel) 2020;9:777. <u>https://doi.org/10.3390/antiox9090777</u>
- 2. Shihadeh A. Investigation of mainstream smoke aerosol of the argileh water pipe. Food Chem Toxicol 2003;41:143-152. <u>https://doi.org/10.1016/S0278-6915(02)00220-X</u>
- Maziak W, Ben Taleb Z, Jawad M, Afifi R, Nakkash R, Akl EA, Ward KD, et al. Consensus statement on assessment of waterpipe smoking in epidemiological studies. Tob Control 2017;26:338-343. <u>https://doi.org/10.1136/tobaccocontrol-2016-052958</u>
- Nemmar A, Al-Salam S, Beegam S, Yuvaraju P, Ali BH. Gum Arabic Ameliorates Impaired Coagulation and Cardiotoxicity Induced by Water-Pipe Smoke Exposure in Mice. Front Physiol 2019;10:53. <u>https://doi.org/10.3389/fphys.2019.00053</u>
- Al Ali R, Vukadinović D, Maziak W, Katmeh L, Schwarz V, Mahfoud F, Laufs U, Böhm M. Cardiovascular effects of waterpipe smoking: a systematic review and meta-analysis. Rev Cardiovasc Med 2020;21:453-468. <u>https://doi.org/10.31083/j.rcm.2020.03.135</u>
- Jukema JB, Bagnasco DE, Jukema RA. Waterpipe smoking: not necessarily less hazardous than cigarette smoking: Possible consequences for (cardiovascular) disease. Neth Heart J 2014;22:91-99. <u>https://doi.org/10.1007/s12471-013-0501-0</u>
- Félétou M. The Endothelium: Part 1: Multiple Functions of the Endothelial Cells-Focus on Endothelium-Derived Vasoactive Mediators. In: *Integrated Systems Physiology: from Molecule to Function to Disease*. San Rafael (CA): Morgan & Claypool Life Sciences Copyright © 2011 by Morgan & Claypool Life Sciences Publishers, 2011. <u>https://doi.org/10.4199/C00031ED1V01Y201105ISP019</u>
- Rammah M, Dandachi F, Salman R, Shihadeh A, El-Sabban M. In vitro effects of waterpipe smoke condensate on endothelial cell function: a potential risk factor for vascular disease. Toxicol Lett 2013;219:133-142. <u>https://doi.org/10.1016/j.toxlet.2013.02.015</u>

- Nemmar A, Al-Salam S, Yuvaraju P, Beegam S, Yasin J, Ali BH. Chronic exposure to water-pipe smoke induces cardiovascular dysfunction in mice. Am J Physiol Heart Circ Physiol 2017;312:H329-H339. <u>https://doi.org/10.1152/ajpheart.00450.2016</u>
- Nemmar A, Beegam S, Zaaba NE, Elzaki O, Pathan A, Ali BH. Waterpipe smoke inhalation induces lung injury and aortic endothelial dysfunction in mice. Physiol Res 2023;72:337-347. <u>https://doi.org/10.33549/physiolres.935042</u>
- Hamadi N, Beegam S, Zaaba NE, Elzaki O, Ali BH, Nemmar A. Comparative Study on the Chronic Vascular Responses Induced by Regular Versus Occasional Waterpipe Smoke Inhalation in Mice. Cell Physiol Biochem 2022;56:13-27. <u>https://doi.org/10.33594/000000491</u>
- Bodas M, Van Westphal C, Carpenter-Thompson R, D KM, Vij N. Nicotine exposure induces bronchial epithelial cell apoptosis and senescence via ROS mediated autophagy-impairment. Free Radic Biol Med 2016;97:441-453. <u>https://doi.org/10.1016/j.freeradbiomed.2016.06.017</u>
- Benedikter BJ, Volgers C, van Eijck PH, Wouters EFM, Savelkoul PHM, Reynaert NL, Haenen G, et al. Cigarette smoke extract induced exosome release is mediated by depletion of exofacial thiols and can be inhibited by thiolantioxidants. Free Radic Biol Med 2017;108:334-344. <u>https://doi.org/10.1016/j.freeradbiomed.2017.03.026</u>
- Al-Azawi A, Sulaiman S, Arafat K, Yasin J, Nemmar A, Attoub S. Impact of Sodium Dichloroacetate Alone and in Combination Therapies on Lung Tumor Growth and Metastasis. Int J Mol Sci 2021;22:12553. <u>https://doi.org/10.3390/ijms222212553</u>
- 15. Allen M, Millett P, Dawes E, Rushton N. Lactate dehydrogenase activity as a rapid and sensitive test for the quantification of cell numbers in vitro. Clin Mater 1994;16:189-194. <u>https://doi.org/10.1016/0267-6605(94)90116-3</u>
- Ali BH, Al Moundhri M, Eldin MT, Nemmar A, Al Siyabi S, Annamalai K. Amelioration of cisplatin-induced nephrotoxicity in rats by tetramethylpyrazine, a major constituent of the Chinese herb Ligusticum wallichi. Exp Biol Med (Maywood) 2008;233:891-896. <u>https://doi.org/10.3181/0711-RM-315</u>
- Higashi Y. Roles of Oxidative Stress and Inflammation in Vascular Endothelial Dysfunction-Related Disease. Antioxidants (Basel) 2022;11:1958. <u>https://doi.org/10.3390/antiox11101958</u>
- D'Onofrio N, Prattichizzo F, Martino E, Anastasio C, Mele L, La Grotta R, Sardu C, et al. MiR-27b attenuates mitochondrial oxidative stress and inflammation in endothelial cells. Redox Biol 2023;62:102681. <u>https://doi.org/10.1016/j.redox.2023.102681</u>
- Nemmar A, Al-Salam S, Beegam S, Yuvaraju P, Ali BH. Aortic Oxidative Stress, Inflammation and DNA Damage Following Pulmonary Exposure to Cerium Oxide Nanoparticles in a Rat Model of Vascular Injury. Biomolecules 2019;9:376. <u>https://doi.org/10.3390/biom9080376</u>
- 20. Miller MR, Newby DE. Air pollution and cardiovascular disease: car sick. Cardiovasc Res 2020;116:279-294. https://doi.org/10.1093/cvr/cvz228
- 21. Nemmar A, Hoylaerts MF, Nemery B. Effects of particulate air pollution on hemostasis. Clin Occup Environ Med 2006;5:865-881.
- Nemmar A, Al-Salam S, Beegam S, Zaaba NE, Elzaki O, Ali BH. Waterpipe smoke inhalation potentiates cardiac oxidative stress, inflammation, mitochondrial dysfunction, apoptosis and autophagy in experimental hypertension. Biomed Pharmacother 2023;158:114144. <u>https://doi.org/10.1016/j.biopha.2022.114144</u>
- Aykul S, Martinez-Hackert E. Determination of half-maximal inhibitory concentration using biosensor-based protein interaction analysis. Anal Biochem 2016;508:97-103. <u>https://doi.org/10.1016/j.ab.2016.06.025</u>
- 24. Kopel E, Kivity S, Morag-Koren N, Segev S, Sidi Y. Relation of serum lactate dehydrogenase to coronary artery disease. Am J Cardiol 2012;110:1717-1722. <u>https://doi.org/10.1016/j.amjcard.2012.08.005</u>
- Zaaba NE, Al-Salam S, Beegam S, Elzaki O, Yasin J, Nemmar A. Catalpol Attenuates Oxidative Stress and Inflammation via Mechanisms Involving Sirtuin-1 Activation and NF-κB Inhibition in Experimentally-Induced Chronic Kidney Disease. Nutrients 2023;15:237. <u>https://doi.org/10.3390/nu15010237</u>
- 26. Nemmar A, Al Salam S, Zia S, Dhanasekaran S, Shudadevi M, Ali BH. Time-course effects of systemically administered diesel exhaust particles in rats. Toxicol Lett 2010;194:58-65. <u>https://doi.org/10.1016/j.toxlet.2010.02.001</u>
- Nemmar A, Al-Salam S, Yuvaraju P, Beegam S, Ali BH. Emodin mitigates diesel exhaust particles-induced increase in airway resistance, inflammation and oxidative stress in mice. Respir Physiol Neurobiol 2015;215:51-57. <u>https://doi.org/10.1016/j.resp.2015.05.006</u>

- Al Suleimani YM, Ali BH, Ali H, Manoj P, Almashaiki KS, Abdelrahman AM. The Salutary Effects of Diminazene, Lisinopril or Valsartan on Cisplatin - Induced Acute Kidney Injury in Rats: A Comparative Study. Physiol Res 2024;73:227-237. <u>https://doi.org/10.33549/physiolres.935210</u>
- Yoshida M, Minagawa S, Araya J, Sakamoto T, Hara H, Tsubouchi K, Hosaka Y, et al. Involvement of cigarette smoke-induced epithelial cell ferroptosis in COPD pathogenesis. Nat Commun 2019;10:3145. <u>https://doi.org/10.1038/s41467-019-10991-7</u>
- Slíva J, Charalambous C, Bultas J, Karetová D. A new strategy for the treatment of atherothrombosis inhibition of inflammation. Physiol Res 2019;68(Suppl 1):S17-S30. <u>https://doi.org/10.33549/physiolres.934327</u>
- Rahman A, Kefer J, Bando M, Niles WD, Malik AB. E-selectin expression in human endothelial cells by TNFalpha-induced oxidant generation and NF-kappaB activation. Am J Physiol 1998;275:L533-L544. https://doi.org/10.1152/ajplung.1998.275.3.L533
- Yang Z, Knight CA, Mamerow MM, Vickers K, Penn A, Postlethwait EM, Ballinger SW. Prenatal environmental tobacco smoke exposure promotes adult atherogenesis and mitochondrial damage in apolipoprotein E-/- mice fed a chow diet. Circulation 2004;110:3715-3720. <u>https://doi.org/10.1161/01.CIR.0000149747.82157.01</u>
- Fetterman JL, Sammy MJ, Ballinger SW. Mitochondrial toxicity of tobacco smoke and air pollution. Toxicology 2017;391:18-33. <u>https://doi.org/10.1016/j.tox.2017.08.002</u>
- Zorova LD, Popkov VA, Plotnikov EY, Silachev DN, Pevzner IB, Jankauskas SS, Babenko VA, et al. Mitochondrial membrane potential. Anal Biochem 2018;552:50-59. <u>https://doi.org/10.1016/j.ab.2017.07.009</u>
- 35. Kuchařová M, Hronek M, Rybáková K, Zadák Z, Štětina R, Josková V, Patková A. Comet assay and its use for evaluating oxidative DNA damage in some pathological states. Physiol Res 2019;68:1-15. <u>https://doi.org/10.33549/physiolres.933901</u>
- Omari Shekaftik S, Nasirzadeh N. 8-Hydroxy-2'-deoxyguanosine (8-OHdG) as a biomarker of oxidative DNA damage induced by occupational exposure to nanomaterials: a systematic review. Nanotoxicology 2021;15:850-864. https://doi.org/10.1080/17435390.2021.1936254
- Pourahmad J, Aghvami M, Zarei MH, Naserzadeh P. Cigarette Smoke and Mitochondrial Damage. In: Mitochondrial Dysfunction Caused by Drugs and Environmental Toxicants. WILL E, DYKENS JE (eds), Wiley Online Library, 2018, pp 709-725. <u>https://doi.org/10.1002/9781119329725.ch45</u>
- Brentnall M, Rodriguez-Menocal L, De Guevara RL, Cepero E, Boise LH. Caspase-9, caspase-3 and caspase-7 have distinct roles during intrinsic apoptosis. BMC Cell Biol 2013;14:32. <u>https://doi.org/10.1186/1471-2121-14-32</u>
- Savitskaya MA, Onishchenko GE. Mechanisms of apoptosis. Biochemistry (Moscow) 2015;80:1393-1405. https://doi.org/10.1134/S0006297915110012
- Fitzgerald M-C, O'Halloran PJ, Connolly NMC, Murphy BM. Targeting the apoptosis pathway to treat tumours of the paediatric nervous system. Cell Death Dis 2022;13:460. <u>https://doi.org/10.1038/s41419-022-04900-y</u>
- Wang J, Wilcken DE, Wang XL. Cigarette smoke activates caspase-3 to induce apoptosis of human umbilical venous endothelial cells. Mol Genet Metab 2001;72:82-88. <u>https://doi.org/10.1006/mgme.2000.3115</u>
- 42. Messner B, Frotschnig S, Steinacher-Nigisch A, Winter B, Eichmair E, Gebetsberger J, Schwaiger S, et al. Apoptosis and necrosis: two different outcomes of cigarette smoke condensate-induced endothelial cell death. Cell Death Dis 2012;3:e424. <u>https://doi.org/10.1038/cddis.2012.162</u>
- Zhang C, Qin S, Qin L, Liu L, Sun W, Li X, Li N, Wu R, Wang X. Cigarette smoke extract-induced p120mediated NF-κB activation in human epithelial cells is dependent on the RhoA/ROCK pathway. Sci Rep 2016;6:23131. <u>https://doi.org/10.1038/srep23131</u>
- 44. Daijo H, Hoshino Y, Kai S, Suzuki K, Nishi K, Matsuo Y, Harada H, Hirota K. Cigarette smoke reversibly activates hypoxia-inducible factor 1 in a reactive oxygen species-dependent manner. Sci Rep 2016;6:34424. https://doi.org/10.1038/srep34424
- 45. Fitzpatrick SF, Tambuwala MM, Bruning U, Schaible B, Scholz CC, Byrne A, O'Connor A, et al. An intact canonical NF-κB pathway is required for inflammatory gene expression in response to hypoxia. J Immunol 2011;186:1091-1096. <u>https://doi.org/10.4049/jimmunol.1002256</u>