Cardiovascular effects of nose-only water-pipe smoking exposure in mice

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Nemmar A, Yuvaraju P, Beegam S, John A, Raza H, Ali BH. Cardiovascular effects of nose-only water-pipe smoking exposure in mice. Am J Physiol Heart Circ Physiol 305: H740–H746, 2013. First published June 28, 2013; doi:10.1152/ajpheart.00200.2013.—Water-pipe smoking (WPS) is a major type of smoking in Middle Eastern countries and is increasing in popularity in Western countries and is perceived as relatively safe. However, data on the adverse cardiovascular effects of WPS are scarce. Here, we assessed the cardiovascular effects of nose-only exposure to mainstream WPS generated by commercially available honey-flavored “moassel” tobacco in BALB/c mice. The duration of the session was 30 min/day for 1 mo. Control mice were exposed to air. WPS caused a significant increase of systolic blood pressure in vivo (+13 mmHg) and plasma concentrations of IL-6 (+30%) but not that of TNF-α. Heart concentrations of IL-6 (+184%) and TNF-α (+54%) were significantly increased by WPS. Concentrations of ROS (+95%) and lipid peroxidation (+27%) were significantly increased, whereas those of GSH were decreased (−21%). WPS significantly shortened the thrombotic occlusion time in pial arterioles (−46%) and venules (40%). Plasma von Willebrand factor concentrations were significantly increased (+14%) by WPS. Erythrocyte numbers (+15%) and hematocrit (+17%) were significantly increased. Blood samples taken from mice exposed to WPS and exposed to ADP showed significant platelet aggregation compared with air-exposed mice. WPS caused a significant shortening of activated partial thromboplastin time (−45%) and prothrombin time (−13%). We conclude that 1-mo nose-only exposure to WPS increased SBP and caused cardiac inflammation, oxidative stress, and prothrombotic events. Our findings provide plausible elucidation that WPS is injurious to the cardiovascular system.

water-pipe smoking; nose-only exposure; heart; oxidative stress; inflammation; thrombosis

WATER-PIPE (also called Hubble bubble, shisha, hookah, or narghile) smoking (WPS) is a method of tobacco smoking commonly practiced in the Middle East, India, and China and is now increasing all over the world, including in Western countries (9, 17, 21, 23). In fact, in the United States, there are ~300 WPS bars and cafés, located in two-thirds of the states, usually near colleges and universities (8). There is evidence that those who are occasional or regular water-pipe smokers are more likely to become regular cigarette smokers, suggesting that WPS may be a potential entryway for regular cigarette use (16). Despite its widespread use, few studies to date have documented the adverse cardiopulmonary consequences of WPS (17). In WPS, smoke passes through water before inhalation by the smokers (23). The tobacco used in WPS is either a pure unflavored tobacco, in which case it is known as “agamy,” or a fruit-flavored tobacco, which is usually prepared by adding honey, glycerin, and other flavors with mild aromatic smoke, known as “maassel” (32). The tobacco used in WPS (“moassel”) typically weighs 10–20 g and contains 30% tobacco and 70% honey or molasses. Most smoking sessions last 30 min or up to several hours (9). WPS contains harmful constituents including nicotine, carbon monoxide, carcinogens, tar, and heavy metals (35). Estimates of the equivalence between cigarette smoking (CS) and WPS varies between 2 and 10 cigarettes for occasional and daily WPS, respectively, and 100 cigarettes for 200 puffs/WPS session (24, 39).

While the adverse health effects related to CS have been extensively described, data on the cardiovascular effects of WPS are very scarce (17). Al-Kubati et al. (1) demonstrated that WPS induced a high increase in heart rate, systolic blood pressure (SBP), diastolic blood pressure, and mean blood pressure and markedly impaired baroreflex sensitivity in healthy normotensive subjects. More recently, Hakim et al. (13) reported that one session of WPS resulted in significant increases in carboxyhemoglobin concentrations, SBP, diastolic blood pressure, heart rate, and respiratory rate. Clinical studies have reported difficulties in studying the isolated effects of WPS because most of the smokers are also current or past cigarette smokers. Therefore, experimental studies investigating the pathophysiological mechanisms underlying the cardiovascular adverse effect of WPS are critical and much needed.

Consequently, the purpose of this study was to examine the effect of exposure to WPS for 1 mo in mice using a relevant exposure system, viz. nose-only exposure, on cardiovascular parameters, including 1) SBP, 2) concentrations of proinflammatory cytokines and markers of oxidative stress in the heart, 3) thrombosis in pial arterioles and venules in vivo, and 4) platelet aggregation in whole blood and activation of coagulation in vitro.

MATERIALS AND METHODS

Animals and Treatments

This project was reviewed and approved (A7–12; Apr. 8, 2012) by the Institutional Review Board of the United Arab Emirates University (College of Medicine and Health Sciences), and experiments were performed in accordance with protocols approved by the Institutional Animal Care and Research Advisory Committee.

WPS Exposure

BALB/C mice (Taconic Farms, Germantown, NY) were housed in a conventional animal house and maintained on a 12:12-h light-dark
cycle (lights on at 6:00 AM). Animals were placed in cages and supplied with pelleted food and water ad libitum. After 1 wk of acclimatization, animals were randomly divided into air (control) and WPS-exposed groups. Mice were placed in soft restraints and connected to the exposure tower (29, 30, 33). They were exposed to WPS or air through their noses using a nose-only exposure system (InExpose System, Scireq). Animals were exposed to mainstream WPS generated by commercially available honey-flavored moassel tobacco (Al Fakher, Ajman, United Arab Emirates). The tobacco was lit with an instant light charcoal disk (Star, 3.5-cm diameter and 1-cm width). A standard of 1 puff of 2-s duration was taken once a minute followed by an application of 58 s of fresh air at a rate of 6 ml/s. The duration of the session was 30 min/day for 1 mo (except during the days of Friday and Saturday each week). At the end of the 1-mo exposure period, various cardiovascular end points were measured.

**SBP Measurement**

SBP was measured using a computerized noninvasive tail-cuff manometry system (AD Instruments, Colorado Springs, CO). To avoid procedure-induced anxiety, mice were trained for 3 consecutive days before the experimental procedures.

**Blood Collection and Cell Count**

After SBP measurements, the same animals were anesthetized intraperitoneally with pentobarbital sodium (45 mg/kg), and blood was then drawn from the inferior vena cava in EDTA (4%). A sample was used for platelet and red blood cell counts and hematocrit determination using an ABX VET ABC hematology analyzer with a mouse card (ABX Diagnostics, Montpellier, France). The remaining blood was centrifuged for 15 min at 4°C at 900 g, and the plasma samples obtained were stored at −80°C pending analysis.

**Measurements of IL-6 and TNF-α in the Heart and Plasma**

At the end of the 1-mo exposure period to WPS or air, animals were euthanized by an overdose of pentobarbital sodium, and their hearts were quickly collected and rinsed with ice-cold PBS (pH 7.4) before homogenization in 50 mM Tris buffer (pH 7.4) containing 400 mM NaCl and 0.5% Triton X-100 at 4°C (6). Homogenates were centrifuged for 10 min at 3,000 g to remove cellular debris, and supernatants were used for further analysis. Protein content was measured by Bradford’s method as previously described (29, 30). Concentrations of IL-6 and TNF-α in the heart and plasma were determined using ELISA kits (Duo Set, R&D Systems, Minneapolis, MN).

**Measurement of Markers of Oxidative Stress in the Heart**

In a separate group of mice, at the end of the 1-mo exposure period to WPS or air, animals were euthanized by an overdose of pentobarbital sodium, and their hearts were quickly collected and rinsed with ice-cold PBS (pH 7.4) before homogenization in 0.1 M phosphate buffer (pH 7.4) containing 0.15 M KCl, 0.1 mM EDTA, 1 mM DTT, and 0.1 mM PMSF at 4°C. Homogenates were centrifuged for 10 min at 3,000 g to remove cellular debris, and supernatants were used for further analysis. Protein content was measured by Bradford’s method as previously described (29, 30).

**Measurement of ROS and lipid peroxidation.** ROS were measured in whole cardiac tissue homogenates, which were obtained as described above, using 2′,7′-dichlorofluorescein diacetate (Molecular Probes, Eugene, OR) as a fluorescent probe as previously described (29, 30). The results were normalized as ROS produced per milligram of protein. NADPH-dependent membrane lipid peroxidation (LPO) was measured as thiobarbituric acid-reactive substances using malondialdehyde as the standard (Sigma-Aldrich, St. Louis, MO) (29, 30).

**Measurement of GSH concentrations.** Measurement of GSH concentrations was carried out in control and WPS-exposed animals by standard procedures as previously described (29, 30).

**Measurement of SOD activity.** SOD activity was measured as the conversion of nitroblue tetrazolium (NBT) to NBT-diformazan according to the vendor’s protocol (R&D Systems). The extent of reduction in the appearance of NBT-diformazan was used as a measure of SOD activity present in the heart (29, 30).

**Experimental Pial Cerebral Arteriolar Thrombosis Model**

In a separate experiment, in vivo pial arteriolar and venular thrombogenesis was measured after WPS or air exposure, according to a previously described technique (25, 30, 31). Briefly, the trachea was intubated after the induction of anesthesia with urethane (1 mg/g body wt ip), and a 2-Fr venous catheter (Portex, Hythe, UK) was inserted in the right jugular vein for the administration of fluorescein (Sigma-Aldrich). Thereafter, a craniotomy was first performed on the left side, using a microdrill, and the dura was stripped open. Only untraumatized preparations were used, and those showing trauma to either the microvessels or underlying brain tissue were discarded. Animals were then placed on the stage of a fluorescence microscope (Olympus, Melville, NY) attached to a camera and DVD recorder. At 37°C, as monitored by a rectal thermoprobe connected to a temperature reader (Physitemp Instruments). The cranial preparation was moistened continuously with artificial cerebrospinal fluid of the following composition (in mM): 124 NaCl, 5 KCl, 3 NaH2PO4, 2.5 CaCl, 4 MgSO4, 23 NaHCO3, and 10 glucose (pH 7.3–7.4). A field containing arterioles and venules of 15–20 μm in diameter was chosen. Such a field was taped before and during the photochemical insult. The photochemical insult was carried out by injecting fluorescein (0.1 ml/mouse of 5% solution) via the jugular vein, which was allowed to circulate for 30–40 s. The cranial preparation was then exposed to stabilized mercury light. This combination produces endothelial injury of the arterioles and venules. This, in turn, causes platelets to adhere at the site of endothelial damage and then aggregate. The platelet aggregates and thrombus formation grow in size until complete arteriolar or venular occlusion. The time from the photochemical insult until full vascular occlusion (time to flow stop) in arterioles and venules was measured in seconds. At the end of the experiments, animals were euthanized by an overdose of urethane.

**Platelet Aggregation in Mouse Whole Blood**

In mice exposed to WPS or air for 1 mo, a platelet aggregation assay in whole blood was performed with slight modifications as previously described (28). After anesthesia, blood from separate animals was withdrawn from the inferior vena cava and placed in citrate (3.8%), and 100-μl aliquots were added to the well of a Merlin coagulometer (MC 1 VET, Merlin, Lemgo, Germany). Blood samples were incubated at 37.2°C with ADP (1 μM) for 3 min and then stirred for another 3 min. At the end of this period, 25-μl samples were removed and fixed on ice in 225 ml cellFix (Becton Dickinson, Franklin Lakes, NJ). After fixation, single platelets were counted in a VET ABX Micros with a mouse card (ABX). The occurrence of platelet aggregation induced by ADP caused a decrease in the counted single platelets in the blood obtained from WPS- or air-exposed mice compared with untreated (without ADP) whole blood obtained from control (unexposed) mice. Therefore, the degree of platelet aggregation after WPS or air exposure was expressed as a percentage of that obtained in untreated whole blood obtained from control mice.

**Prothrombin Time and Activated Partial Thromboplastin Time Measurements in Plasma In Vitro**

On separate animals, at the end of the 1-mo exposure period to WPS or air, blood was withdrawn from mice as described above. The prothrombin time (PT) was measured (26, 31) on freshly collected platelet-poor plasma with human relipidated recombinant thromboplastin (Recombiplastin, Instrumentation Laboratory, Orangeburg, NY) in combination with a Merlin coagulometer (MC 1 VET, Merlin). The activated...
partial thromboplastin time (aPTT) was measured (26, 31) with automated aPTT reagent from bioMerieux (Durham, NC) using a Merlin coagulometer (MC 1 VET, Merlin). Normal plasma used as the reference for both PT and aPTT was prepared by pooling equal portions of platelet-poor plasmas from the blood of six untreated mice.

Measurement of Von Willebrand Factor in Plasma

The plasma concentration of von Willebrand factor (vWF) was measured using an ELISA kit (Uscn, Life Science, Wuhan, China).

Statistics

All statistical analyses were performed with GraphPad Prism software (version 5). To determine whether parameters were normally distributed, the D’Agostino and Pearson normality test was applied. Normally distributed data were analyzed using an unpaired \( t \)-test for differences between groups. All data in figures are reported as means ± SE. \( P \) values of \( \leq 0.05 \) were considered significant.

RESULTS

Effect of WPS on SBP

Figure 1 shows the effect of WPS or air exposure on SBP in mice. SBP in animals exposed to WPS (125 ± 6 mmHg, \( P < 0.05 \)) was significantly higher than that in mice exposed to air (112 ± 2 mmHg).

Effect of WPS on IL-6 and TNF-\( \alpha \) Concentrations in the Heart and Plasma

Figure 2 shows the effect of WPS or air exposure on concentration of IL-6 and TNF-\( \alpha \) in the heart and plasma. After the exposure to WPS, the concentration of IL-6 in the heart was significantly higher than that in mice exposed to air (Fig. 2A). Likewise, mice exposed to WPS showed a significantly higher concentration of TNF-\( \alpha \) in the heart compared with air-exposed mice (Fig. 2B). WPS exposure caused a significant increase of IL-6 concentrations in plasma (Fig. 2C). However, at the time point investigated, plasma concentrations of TNF-\( \alpha \) were not affected (Fig. 2D).

Effect of WPS on ROS, LPO, GSH, and SOD Levels in the Heart

After exposure to WPS, ROS concentrations in the heart significantly increased compared with air exposure (Fig. 3A). Similarly, WPS exposure caused a significant elevation in LPO concentrations in the heart compared with the air-exposed group, suggesting an increase in oxidative stress (Fig. 3B). Figure 3C shows the effect of WPS or air exposure on reduced GSH concentration. WPS exposure caused a significant decrease of GSH concentrations compared with the air-exposed group, suggesting depletion of the antioxidant GSH (Fig. 3C). Nevertheless, a slight but insignificant decrease of SOD activity in the heart was observed 1 mo after exposure to WPS compared with the air-exposed group (Fig. 3D).

Effect of WPS on Platelet and Erythrocyte Numbers and Hematocrit

Figure 4 shows the effect of WPS on platelet and erythrocyte numbers and hematocrit. In WPS-exposed animals, we found a slight but insignificant increase of platelet numbers compared...
with the air-exposed group (Fig. 4A). However, numbers of erythrocytes were significantly increased in WPS-exposed mice compared with those exposed to air \((P < 0.05)\). Likewise, hematocrit was significantly increased in WPS-exposed mice compared with air-exposed mice \((P < 0.05)\).

**Effect of WPS on Photochemically Induced Thrombosis in Pial Arterioles and Venules**

Nose-only exposure to WPS for 1 mo induced a shortening of the occlusion time in pial arterioles in a photochemically injured vessel (Fig. 5A) compared with air-exposed mice. Similarly to pial arterioles, WPS exposure caused a prothrombotic tendency in pial venules, which was reflected by a significant shortening of the occlusion time compared with the air-exposed group (Fig. 5B).

**Effect of WPS on vWF Concentrations in Plasma**

vWF concentrations in plasma were significantly increased \((P < 0.005)\) in mice after WPS exposure compared with those exposed to air (Fig. 6).

**Effect of WPS on Platelet Aggregation in Whole Blood In Vitro**

Whole blood collected from mice after nose-only exposure to WPS and exposure to ADP \((1 \mu M)\) caused significant \((P < 0.0001)\) platelet aggregation compared with blood obtained from mice exposed to air (Fig. 7).

**Effect of WPS on PT and aPTT In Vitro**

Figure 8 shows the effect of 1-mo nose-only exposure to WPS or air on PT and aPTT in mouse plasma. The shortening of PT and aPTT reflects hypercoagulability. Exposure to WPS induced a significant \((P < 0.0001)\) shortening of PT compared with plasma obtained from air-exposed mice (Fig. 7A). In the same way, aPTT was also significantly \((P < 0.01)\) reduced in plasma obtained from WPS-exposed mice compared with that taken from mice exposed to air.

**DISCUSSION**

The present study provides evidence showing that nose-only exposure to WPS for 1 mo induces an increase in SBP and causes inflammation and oxidative stress in the heart and prothrombotic and hypercoagulability effects in vivo and in vitro. Despite the perception that the risks of the WPS may be less than those of cigarettes, a recent report has suggested that its harmful effects are similar to those of cigarettes and that the water-pipe may be a bridge to CS. The highest prevalence of use of WPS (with up to 34% reported) is currently among adolescents and women (12, 22). The spread of WPS to

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**Fig. 3.** ROS (A), lipid peroxidation (LPO; B), GSH (C), and SOD (D) levels in the hearts of mice at the end of the 1-mo exposure period to air (control) or WPS. Data are means ± SE; \(n = 8\). *\(P < 0.05\), **\(P = 0.01\), and ***\(P < 0.0001\) compared with the corresponding air-exposed group.

**Fig. 4.** Platelet (A) and erythrocyte (B) numbers and hematocrit (C) at the end of the 1-mo exposure period to air (control) or WPS in mice. Data are means ± SE; \(n = 8\). *\(P < 0.05\) compared with the corresponding air-exposed group.
Western countries, notably among youth, poses a new threat to the public campaign against smoking. It has been estimated that 20–40% of college students in the United States have experienced WPS (11). In one British university, it was found that 8% of students were regular water-pipe smokers, with both ever and regular smoking becoming more prevalent with time at the university. WPS is common among students of nearly all ethnic backgrounds (15). A recent study (2) from the Gulf Registry of Acute Coronary Events, which included 6,701 consecutive acute coronary patients in Bahrain, Kuwait, Qatar, Oman, United Arab Emirates, and Yemen, found that 38% of patients registered were cigarette smokers and 4.4% were water-pipe smokers. The study also found that the water-pipe smokers were older than the cigarette smokers and more likely to be women (2).

In the present study, we used nose-only exposure to WPS in mice. It has been previously shown that this animal model most likely best resembles the human exposure situation (33, 36). Since a large number of experimental and clinical studies has been published on the adverse cardiovascular effects of CS, the present study was specifically designed to assess the cardiac and thrombotic effects of WPS. Our data show that nose-only exposure to WPS for 1 mo causes a significant increase in SBP. Our finding corroborates those of recent clinical studies (1, 13, 34), which reported an increase in blood pressure and heart rate after exposure to WPS in healthy subjects. These hemodynamic changes were suggested to be mediated by nicotine, which activates the sympathetic nervous system with a subse-

![Fig. 5. Thrombotic occlusion time in pial arterioles (A) and venules (B) at the end of the 1-mo exposure period to air (control) or WPS in mice. Data are means ± SE; n = 8. *P < 0.0001 compared with the corresponding air-exposed group.](image)

![Fig. 6. von Willebrand Factor (vWF) plasma levels at the end of the 1-mo exposure period to air (control) or WPS in mice. Data are means ± SE; n = 8. *P < 0.005 compared with the corresponding air-exposed group.](image)

![Fig. 7. In vitro platelet aggregation in whole blood at the end of the 1-mo exposure period to air (control) or WPS in mice. Blood samples obtained from mice exposed either to air or WPS for 1 mo were incubated at 37.2°C with ADP (1 μM) for 3 min and stirred for another 3 min, and single platelets were then counted. The degree of platelet aggregation after WPS or air exposure was expressed as a percentage of that obtained in untreated (without ADP) whole blood obtained from control (unexposed) mice. Data are means ± SE; n = 5. *P < 0.001 compared with the corresponding air-exposed group.](image)

![Fig. 8. Prothrombin time (PT; A) and activated partial thromboplastin time (aPTT; B) at the end of the 1-mo exposure period to air (control) or WPS in mice. Data are means ± SE; n = 8. *P < 0.01 and **P < 0.0001 compared with the corresponding air-exposed group.](image)
quent release of norepinephrine, epinephrine, and vasopressin, or by nicotine’s direct effect on the endothelium (34). Excess sympathetic stimulation in cigarette smokers contributes to cardiovascular morbidity and mortality (34). The effect of WPS on SBP that we observed can be related to the oxidative stress and inflammation observed in heart. Indeed, we found a significant increase of ROS and LPO after exposure to WPS. Moreover, our data confirm that WPS decreases heart GSH concentrations, leaving heart tissues vulnerable to damage by oxygen free radicals. SOD activity was slightly and insignificantly affected by WPS exposure. This may suggest that cardiac SOD efficiency to reduce superoxide radicals is not appreciably affected by WPS. We (29, 30) have recently demonstrated that short-term nose-only exposure to CS causes oxidative stress in various organs, including the heart, lung, kidney, and liver. Furthermore, we found a significant increase of both TNF-α and IL-6 in the heart. Also, IL-6 levels in plasma significantly increased after WPS exposure. However, the absence of an increase of TNF-α at the 1-mo exposure time point does not necessarily exclude its possible release at an earlier time point. Additional experiments are needed to study the kinetics of release of TNF-α in plasma after WPS. Enhanced circulating concentrations of IL-6 and TNF-α have been reported in experimental animals exposed to CS (41) and patients with chronic obstructive pulmonary disease (19). ROS can activate redox-sensitive transcription factors, nuclear factor-κB, and activator protein-1, activating the genes of proinflammatory mediators TNF-α, IL-1β, and IL-6 (20). The latter proinflammatory cytokines play an important role in the pathogenesis of atherosclerosis and were detected in plasma and the myocardium of patients with heart failure (4, 14).

We found a significant increase in erythrocyte numbers after WPS exposure. This finding is suggestive of a bone marrow response, causing an increase in red blood cells. A consistent link between inhalation of pollutants and the bone marrow response has previously been reported, indicating a strong link between lung and systemic events (37). The elevation in erythrocytes and hematocrit observed in the present study corroborate previous findings that showed a rise of erythrocytes and hematocrit in young male smokers (18). Augmented erythrocyte counts, hematocrit, blood viscosity, and the ongoing inflammatory process potentiate the prothrombotic process associated with CS exposure (3).

Our results show that 1-mo nose-only WPS exposure promotes pial arteriolar and venular thrombosis in a photochemically injured vessel. These observations show that circulating activated platelets induced by WPS exposure can cause thrombotic events when they encounter damaged endothelium. Such areas of limited endothelial desquamation often form the nidus of a platelet thrombus as they uncover subendothelial collagen and vWF, which promote platelet adhesion and activation. Such superficial erosions may account for approximately one-quarter of fatal coronary thrombosis (38). It has been reported that CS exposure causes alterations in platelet function, anti-thrombotic/prothrombotic factors, and fibrinolytic factors (3). We (25, 27) have recently demonstrated that acute exposure to particulate air pollution causes thrombotic complication in mice. However, short-term (4-days) nose-only exposure to CS did not affect thrombogenesis (30). The discrepancy between the latter finding and the present finding can be related to the exposure duration (4 days for CS and 1 mo for WPS). vWF is synthesized by endothelial cells, stored in the Weibel-Palade bodies, and secreted on endothelial activation (10). Increased plasma vWF levels have been associated with cardiovascular disease (7). The increase of endothelial vWF release after WPS exposure observed in the present study suggests that the prothrombotic tendency developed with endothelial activation. vWF is instrumental in arterial thrombogenesis but has also been implicated in venous thrombogenesis (40). Indeed, it has been previously shown that the murine anti-vWF antibody AjvW-2 is a potent inhibitor of shear-induced platelet activation and of vWF-dependent thrombosis (40).

To gain more insights into the mechanism underlying the in vivo prothrombotic effects of WPS, we performed additional in vitro experiments where we assessed the effects of WPS on platelet aggregation in whole blood and the activation of coagulation, i.e., PT and aPTT. Indeed, we assessed the in vitro effects of ADP on platelet aggregation in whole blood collected from mice exposed either to WPS or air. Our data show the occurrence of platelet aggregation in the blood of WPS-exposed mice compared with that of air-exposed mice. The latter finding suggests a priming of platelet activation and their contribution in the development of a thrombotic tendency, when such primed platelets encounter a (mildly) injured vessel wall, as observed in vivo in the present study. Moreover, we found an activation of coagulation because both PT and aPTT were activated in vitro. PT measures the formation of the fibrin clot through the activity of the extrinsic and common coagulation pathways, which involve the interaction of tissue factor and activated factor VII, in addition to factor X, factor V, prothrombin, and fibrinogen. aPTT, in contrast to PT, measures the activity of the intrinsic and common pathways of coagulation. The shortening of PT and aPTT reflects hypercoagulability. Our results corroborate findings that showed that exposure to air pollution is associated with changes in the global coagulation function, suggesting a tendency toward hypercoagulability (5, 26, 31).

We conclude that nose-only exposure to WPS for 1 mo causes an increase in SBP, induces inflammation and oxidative stress in the heart, and causes prothrombotic and hypercoagulability effects in vivo and in vitro. Additional studies are required to further assess the effects of WPS and to compare the short-term and long-term effects of WPS and study the effects of additives that are supplemented to tobacco added in shisha (e.g., fruit essences, sweeteners, and other unknown substances). Our findings provide biological plausibility on the detrimental effect of WPS on the cardiovascular system.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
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