



# Effects of Endurance Exercise Training on Cardiac Dysfunction Induced by Waterpipe Tobacco Smoking

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## Original Article

### Abstract

**Background:** There is an increasing popularity of waterpipe tobacco smoking (WTS) in youth and even in athletes worldwide. Despite the existence of evidence of the harmful effects of hookah smoke on various systems of the body, especially the cardiovascular system, its simultaneous effect with exercise training has not been well studied. We assessed the effects of WTS exposure with/without swimming exercise on blood pressure (BP), and heart histology and mechanical performance in male Wistar rats.

**Methods:** The animals were divided into 4 groups of sedentary control (CTL), waterpipe tobacco smoking (S), mild endurance swimming exercise training (Ex), and waterpipe smoking plus exercise (S + Ex). The duration of WTS and exercise was 8 weeks.

**Findings:** BP and heart rate (HR) did not show a significant difference among the groups. WTS increased the TNF- $\alpha$  level of the heart ( $P < 0.05$  vs. CTL) and cardiac tissue lesions ( $P < 0.05$  vs. CTL), and reduced +dp/dt max, -dp/dt max, and heart contractility indices ( $P < 0.01$ ,  $P < 0.01$ , and  $P < 0.05$ , respectively, vs. CTL and Ex groups). It also increased the Tau index ( $P < 0.05$  vs. CTL;  $P < 0.01$  vs. Ex groups) of the left ventricle. However, the combination of exercise and WTS reduced the TNF- $\alpha$  level, improved the heart activity of superoxide dismutase (SOD) and catalase enzymes, and prevented the negative effects of smoking on heart function and morphology.

**Conclusion:** Mild exercise prevents WTS-induced left ventricular systolic and diastolic dysfunction partly via improvement of antioxidants and attenuation of pro-inflammatory cytokines.

**Keywords:** Smoking water pipes; Exercise training; Left ventricular function; Antioxidants; Cytokines

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## Introduction

Waterpipe (Hookah, Shisha, Ghalyan, Narghile, Arghile, Goza, Narkeela, or Hubble-bubble) tobacco smoking is a non-cigarette form of tobacco use that is becoming more prevalent worldwide. Waterpipe tobacco smoking (WTS) or Waterpipe smoking (WPS) is an old method of tobacco smoking in which prior to inhalation by the user, tobacco smoke is passed through a half-full water bowl. First, the sweetened and flavored tobacco mixture (ma'assel) is placed in the ceramic head. The ceramic head is placed atop a conduit that is submerged in a water bowl with a hose attached to it (Figure 1). Usually, charcoal is used to heat the tobacco. Through sucking on the mouthpiece connected to the hose, negative pressure is formed in the bowl and air is drawn over the charcoal. The inhalation of the user causes the air to pass through hot charcoal and then tobacco (a perforated aluminum foil often separates charcoal from tobacco), and the smoke of the charcoal and tobacco to flow to the water and then to the consumer's lungs through the hose.<sup>1</sup>

WTS has become a new strain in the global tobacco epidemic and a public health concern. According to statistics reported by the World Health Organization (WHO), about 2.4 billion people in the world are tobacco users. It was also reported that in 2015 about 6.4 million of deaths were due to tobacco use, which will reach about 8.3 million by 2030 and 1 billion people by the 21<sup>st</sup> century.<sup>2</sup> The prevalence of hookah use in Iran is 11.4% (21.4% in men and 1.4% in women).<sup>3</sup> The prevalence of hookah consumption in Middle Eastern countries is estimated at 6% to 34% of the population of these countries, with higher rates among adolescents. It was reported that mortality rate due to cardiovascular disease (CVD) is 1.96 times higher in WT users than nonsmokers, and the prevalence of coronary disease is higher among WT users than nonsmokers.<sup>4,5</sup>

Increase in heart rate (HR) and blood pressure (BP), decrease in the sensitivity of baroreceptors controlling BP, and changes in hematological parameters following acute WTS were reported.<sup>2,6</sup> Considering the beneficial effects of endurance exercise on the cardiovascular system,<sup>7</sup> some tobacco smokers believe that the combination of use of hookah smoke with exercise can improve its negative cardiovascular effects. Hence, despite

the lack of information on the possible cardiovascular side effects of hookah with exercise, and whether the hookah side effects differ in athletes compared to non-athletes, the prevalence of hookah consumption is increasing in sports communities.<sup>8</sup> Therefore, to clarify this issue, in the present study, the effects of simultaneous hookah consumption and endurance exercise training were investigated on some cardiac performance indicators and BP in a controlled animal model.

## Methods

This study was conducted according to the national guide for the care and use of laboratory animals and the experimental protocol was approved by the Ethics Committee of Kerman University of Medical Sciences (Permission no.: EC/96-10/KNRC). Cotinine kit (Sigma-Aldrich, UK), thiopental sodium (Sandoz, Austria), and double apple flavor tobacco (Al Fakher, Ajman, UAE) were purchased. Catalase activity and lipid peroxidation assay kits were obtained from Navand Salamat Company (Iran). Rat interleukin-6 (IL-6), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-10 and IL-1 $\beta$  enzyme-linked immunosorbent assay (ELISA) kits were purchased from Hangzhou Eastbiopharm Co., LTD (China). Moreover, rat superoxide dismutase (SOD) and glutathione peroxidase (GPx) ELISA Kits were purchased from Shanghai Crystal Day Biotech Co. (China).

Twenty-eight male Wistar rats weighing 200-250 g were randomly divided into 4 groups. The sedentary control (CTL) group was exposed to room air during the study period, exercise training (Ex) group was subjected to 8 weeks of swimming exercise, sedentary Waterpipe tobacco smoking (S) group was exposed to tobacco smoking (30 minutes/day) as explained below, and exercise plus Waterpipe tobacco smoking (S + Ex) group was subjected to both smoking and exercise training. The animals were kept at  $22 \pm 2$  °C with 12-hour light/dark cycle and had access to tap water and standard feeding throughout the period of the experiment.

**Smoke machine and smoking exposure:** An inhalation Plexiglas chamber with the dimensions of 50 (length) \* 30 (width) \* 12 (height) cm was prepared. The smoking machine (Figure 1) used in this study was designed and made in our lab based on previous studies.<sup>2,9</sup> A time regulator

controlled the sequence of smoke puffs and fresh air into and out of the chamber through valves. The smoking ventilation cycle was 3 consecutive 30 seconds; for 30 seconds tobacco smoke was released into the chamber, then the smoke valve was closed and the fresh air inlet was opened for 30 seconds to clear out the chamber, and finally the animals were allowed to inhale fresh air for a period of 30 seconds. This cycle was repeated 20 times ( $20 \times 1.5$ -minute exposure = 30-minute exposure session) for S and S + Ex groups daily. The level of CO concentration was maintained at a similar level [mean  $\pm$  standard deviation (SD):  $886 \pm 103$  ppm] during all of the exposure sessions using Testo 310 measurement device (Testo Ltd., Germany). Sedentary rats were placed in the chamber for 10 minutes, twice a week, to simulate the environmental stress of the protocol. Al Fakher Double Apple tobacco is a well-known brand worldwide and is consumed extensively in Iran. Previously, Schubert et al. measured the average aromatic compounds of flavored waterpipe tobaccos and the sum of volatile flavor substances, including ethyl 2-methyl butyrate, hexanal, limonene, 1-hexanol, cis-3-hexen-1-ol, benzaldehyde, linalool, L-menthol, benzyl acetate, trans-anethole, and benzyl alcohol, was about 500 mg/g tobacco for Al Fakher Double Apple tobacco.<sup>10</sup>

**Exercise training:** The animals were trained with low to moderate swimming endurance training. The swimming pool and exercise protocol was adapted based on a previous study.<sup>11</sup> In brief, a pool of 50 (depth)  $\times$  120 (length) cm and containing warm water (30-32 °C) was used for swimming training of the animals. Exercise training was performed 5 days/week. In the first week, training was conducted for 20 minutes on the first day and was lengthened by 10 minutes every following day; thus, the last day of swimming lasted 60 minutes. During the other weeks, the exercise duration was kept constant. The animals carried a caudal dumbbell that weighed 2% of their body weight during swimming. The weight of the dumbbell was gradually increased to 5% of their body weight on the sixth week and the dumbbell was kept on them constantly from that point on. This protocol is considered as a low-intensity, long training period. Sedentary rats were placed in the pool with low level of water to simulate the water stress of the protocol.



**Figure 1.** Waterpipe tobacco smoking setup

1. The ceramic head of Ghalyan contains tobacco, 2. A Plexiglas chamber (8 mm thick), location of animals for the purpose of inhalation of tobacco smoke, 3. Fresh waterpipe air input, 4. Fresh waterpipe air output, 5. vacuum pump, 6. Electronic valve, 7. Time controller for the sequence of operation of the pump and valve

**Recording and sampling:** Twenty-four hours after the end of the study period, the animals were anesthetized with sodium thiopental [50 mg/kg intraperitoneal (i.p.)]. In order to maintain spontaneous breathing throughout the hemodynamic recording procedures, the animals' trachea was cannulated. A saline filled catheter (saline with 15 IU/ml heparin) was inserted into the left carotid artery and connected to a pressure transducer of a Power Lab physiographic system (ADInstruments, Australia) for arterial BP recording. The other similar polyethylene catheter was inserted into the left ventricle through the right carotid artery for left ventricular pressures and function indices. After recording, the animals' blood samples were collected, and sera were separated by centrifugation and stored at -80 °C. The hearts of animals were removed and frozen in liquid nitrogen and stored at -80 °C. The stored serum and heart tissues were used for measuring cotinine, oxidants, antioxidants, and ILs. Cotinine is an alkaloid component of tobacco and the dominant metabolite of nicotine, and is used as a

biochemical marker of tobacco smoking. Finally, the animals were sacrificed.

**Hemodynamic and heart function indices:** The mean arterial pressure (MAP) was calculated using the following formula: diastolic arterial pressure + 1.3 (systolic arterial pressure-diastolic arterial pressure). The left ventricular end diastolic pressure (LVEDP), maximal positive of changes in left ventricular pressure (+dP/dt max, a contractility velocity index), contractility index [+max dp/dt divided by pressure (P) at the time of maximum change with the dimension of 1/s], maximum rate of reduction in left ventricular pressure (-dP/dt max, a relaxation velocity index), and Tau (left ventricular relaxation time constant as a relaxation index of the heart) indices were calculated.

**Measurement of cytokines, malondialdehyde (MDA), and antioxidants:** The heart apex was removed for molecular measurement. For the assessment of ILs, 40 mg of heart tissue was homogenized on ice and centrifuged at 3000 rpm for 20 minutes at 4 °C. The concentration of total protein was measured through Bradford method using ELISA kits based on the manufacturer's instructions. SOD and GPx activity were measured according to the manufacturer's instructions. Briefly, 50 mg of heart tissue was homogenized on ice in a suitable amount of PBS (pH = 7.4). The sample was then centrifuged at 3000 rpm for 20 minutes at 4 °C. To measure the activity of enzymes, 40 µl of the supernatant was used. The absorbance of each sample was found to be at wavelengths of less than 450 nm. Catalase activity was estimated based on the reaction of catalase with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and methanol, and the production of formaldehyde. Subsequently, 50-100 mg of each tissue sample was homogenized using cold lysing buffer, and then, centrifuged at 8000 rpm for 10 minutes at 4 °C. For the measurement of catalase activity according to the ELISA kit instructions, 100 µl of supernatant was used. The MDA level of the heart, as an index of lipid peroxidation, was measured based on the concentration of thiobarbituric acid reactive substances (TBARS). Moreover, 50-100 mg of each heart sample was homogenized using 1.5% potassium chloride solution. The homogenate was centrifuged at 1200 rpm for 10 minutes. To measure MDA according to the ELISA kit instructions, 250 µl of

the supernatant was used. Quantitative assessments of IL-1β, TNF-α, IL-10, and IL-6 were conducted using ELISA kits based on the manufacturer's instructions.

**Histological study:** For evaluation of morphological changes, heart tissues were embedded into 10% buffered formalin. Sections of 5 µm thickness were cut and stained with hematoxylin and eosin (H&E). Tissue sections were examined by a pathologist blinded to animal groups. Lesions were classified according to the presence of congestion and interstitial edema, leukocyte infiltration, hypercontraction band and hypereosinophilia, myofibrillar degeneration, and the distribution of lesions (e.g., focal, multifocal, locally extensive, or diffuse), and graded in the range of 0-4 (0 = no change; 1 = minimal; 2 = mild; 3 = moderate; and 4 = marked).

The assumption of normality was assessed using the Shapiro-Wilk test, and the homoscedasticity between groups was evaluated using Levene's test. The differences in quantitative data were assessed using one-way and two-way ANOVA. First, two-way ANOVA was carried out to evaluate the effects of WTS, exercise, and their interactions. When one or both main effects were statistically significant, one-way ANOVA and Tukey's post hoc test were used to determine the differences between the means. Differences in pathological scores were analyzed using Kruskal-Wallis test and Mann-Whitney test.  $P < 0.05$  was considered statistically significant.

## Results

**Hemodynamic and cardiac performance:** Plasma cotinine level significantly increased in WTS groups ( $P < 0.001$  versus non-smoking groups) (Table 1). Two-way ANOVA showed no interaction between smoking and exercise in any of the measured parameters. There was no significant difference in systolic pressure, diastolic pressure, and MAP (Table 1) among animal groups ( $P > 0.05$ ).

Two-way ANOVA revealed clearly the effect of exercise on LVEDP ( $P < 0.05$ ) and the Tau index ( $P < 0.01$ ). Two-way ANOVA and Tukey's post hoc tests showed the main effect of smoking on +dP/dt max ( $P < 0.01$ ), -dp/dt max ( $P < 0.05$ ), and the Tau and contractility index ( $P < 0.05$ ) in the waterpipe tobacco smoking group compared to CTL and Ex groups.



**Table 1.** The values of blood pressure (BP), levels of plasma cotinine, and levels of malondialdehyde (MDA), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase, tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), and interleukins-1  $\beta$  (IL-1 $\beta$ ), 6, and 10 in the heart in the animal groups

Variables	CTL	S	Ex	S + Ex
Cotinine (ng/ml)	8.87 $\pm$ 0.48	23.20 $\pm$ 1.15*	7.43 $\pm$ 0.59	18.97 $\pm$ 0.93**‡
SBP (mmHg)	135 $\pm$ 3	117.0 $\pm$ 8.6	130 $\pm$ 9.3	123.0 $\pm$ 4.8
DBP (mmHg)	104 $\pm$ 2	92.0 $\pm$ 6.5	91 $\pm$ 8.8	90.0 $\pm$ 6.6
MAP (mmHg)	119 $\pm$ 2	105.0 $\pm$ 7.3	110 $\pm$ 9	106.0 $\pm$ 5.6
HR (bpm)	170 $\pm$ 6	186 $\pm$ 15	190 $\pm$ 9	193 $\pm$ 11
MDA (nmol/ $\mu$ g of total protein)	1840 $\pm$ 218	2146 $\pm$ 85	1971 $\pm$ 218	2064 $\pm$ 182
GPX (U/ng of total protein)	10.64 $\pm$ 1.07	11.01 $\pm$ 1.20	12.11 $\pm$ 1.5	10.64 $\pm$ 1.10
SOD ( $\mu$ g/ng of total protein)	0.11 $\pm$ 0.03	0.14 $\pm$ 0.01	0.22 $\pm$ 0.03#	0.18 $\pm$ 0.01
Catalase (nmol/min/mg pro)	0.160 $\pm$ 0.008	0.130 $\pm$ 0.011	0.18 $\pm$ 0.008#	0.180 $\pm$ 0.012#
TNF- $\alpha$ (ng/ $\mu$ g of total protein)	2.19 $\pm$ 0.29	3.89 $\pm$ 0.20***	2.072 $\pm$ 0.40#	2.27 $\pm$ 0.49‡
IL-10 (pg/ng of total protein)	1.68 $\pm$ 0.34	1.74 $\pm$ 0.31	2.06 $\pm$ 0.39	1.98 $\pm$ 0.58
IL-1 $\beta$ (pg/ $\mu$ g of total protein)	13.2 $\pm$ 0.7	15.9 $\pm$ 0.3	13.8 $\pm$ 1.4	14.6 $\pm$ 2.1
IL-6 (ng/ $\mu$ g of total protein)	0.62 $\pm$ 0.10	0.75 $\pm$ 0.10	0.55 $\pm$ 0.11	0.63 $\pm$ 0.12

Values are presented as mean  $\pm$  standard error of mean (SEM). n = 6-7 in each group.

CTL: Control group; S: Waterpipe tobacco smoking group; Ex: Exercise training group; S + Ex: Waterpipe tobacco smoking + exercise training group; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; MAP: Mean arterial pressure; HR: Heart rate; MDA: Malondialdehyde; GPx: Glutathione peroxidase; SOD: Superoxide dismutase; TNF- $\alpha$ : Tumor necrosis factor  $\alpha$ ; IL-10: Interleukin-10; IL-1 $\beta$ : Interleukin-1 $\beta$ ; IL-6: Interleukin-6

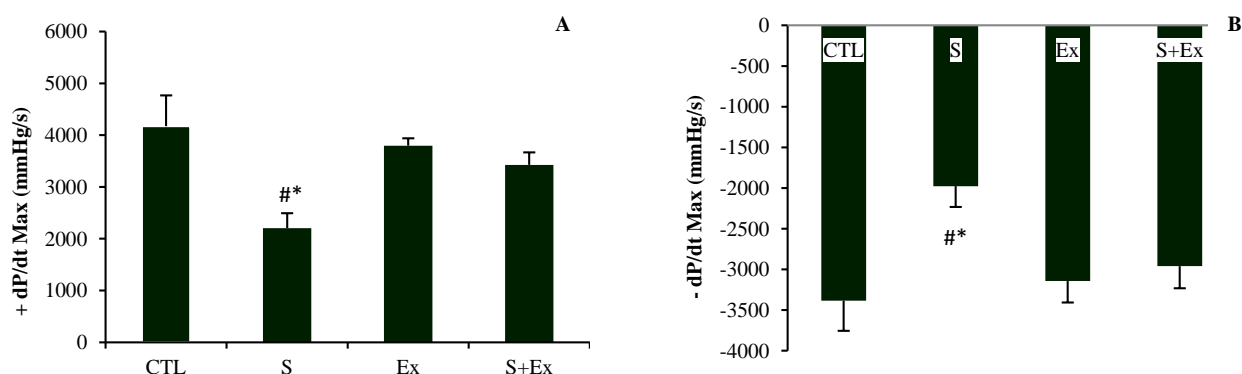
\*P < 0.001 vs. control and exercise groups; \*\*P < 0.001 vs. Waterpipe smoking groups; ‡P < 0.001 vs. other groups; #P < 0.05 vs. CTL and S groups; \*\*\*P < 0.05 vs. CTL; †P < 0.05 vs. S group

However, the combination of exercise and WTS masked these negative effects of smoking so that the S + Ex had no significant difference with CTL and Ex groups (Figures 2, A and B, and 3, A). Moreover, the Tau index significantly increased in the WTS group (P < 0.05 vs. CTL; P < 0.01 vs. Ex group). Exercise training reduced this negative effect of smoking; there was no significant difference in the S + Ex in this regard when compared with Ex and CTL groups (Figure 3, B).

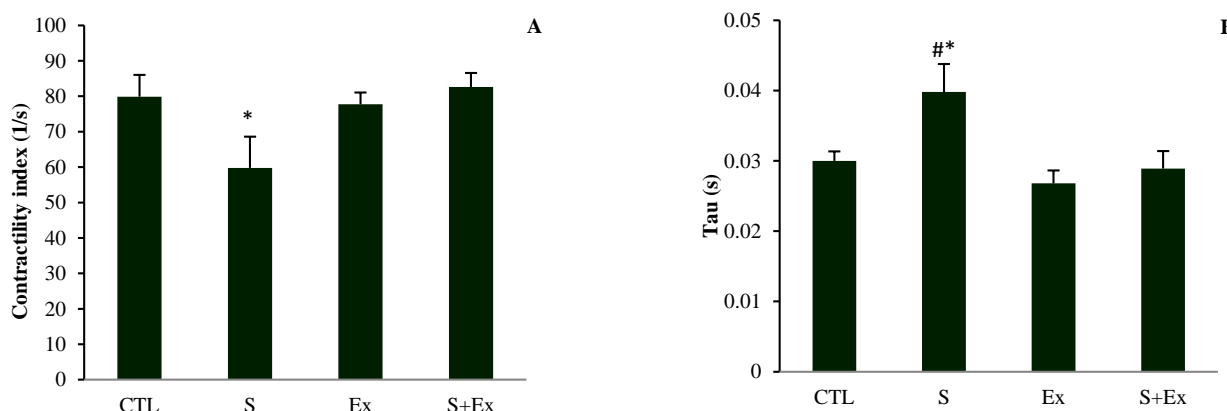
**Cytokines, MDA, GPx, SOD, and catalase of the heart:** WTS significantly increased the TNF- $\alpha$  level

of the heart (P < 0.05 vs. CTL and Ex groups). Swimming training eliminated the effect of smoking on TNF- $\alpha$  of the heart, and there was no significant difference between S + Ex group with CTL and Ex groups (Table 1). The levels of IL-1 $\beta$  and IL-6 were higher in the S group compared with other groups; however, the difference was not significant.

No significant difference was observed in the level of IL-10 between the study groups (Table 1). There was no significant difference in the heart level of MDA and heart GPx activity in the animal groups (Table 1). Exercise alone significantly

**Figure 2.** +dp/dt maximum (Max dp/dt) (A) and -dp/dt maximum (Min dp/dt) (B) in different animal groups (n = 6-7) Values are expressed as mean  $\pm$  SEM.

CTL: Control group; S: Waterpipe tobacco smoking group; Ex: Exercise training group; S + Ex: Waterpipe tobacco smoking + exercise training group, #P < 0.01 vs. CTL and Ex groups; \*P < 0.05 versus S + Ex group



**Figure 3.** Contractility index (A) and Tau index (B) in different animal groups (n = 6-7)

Values are expressed as mean ± SEM.

CTL: Control group; S: Waterpipe tobacco smoking group; Ex: Exercise training group; S + Ex: Waterpipe tobacco smoking + exercise training group; A: \*P < 0.05 vs. CTL group; #P < 0.01 vs. Ex and S + Ex groups; B: \*P < 0.05 vs. other groups

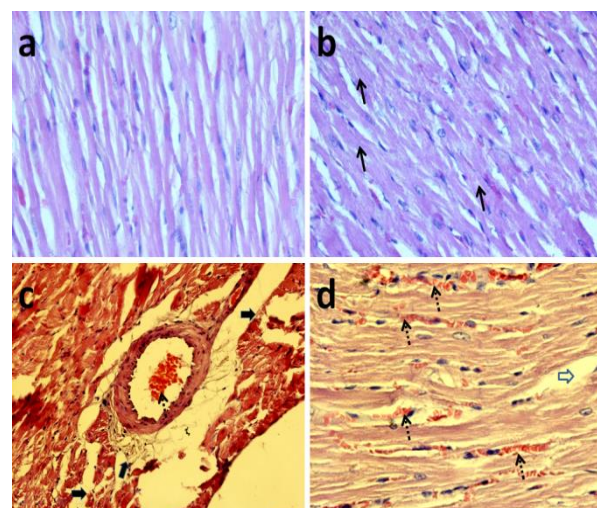
increased the heart SOD and catalase activity (P < 0.05 vs. CTL group). This effect was observed in the combination of exercise and waterpipe smoking, so that the heart catalase activity was also higher in the S + Ex group compared to CTL and S groups (P < 0.05) (Table 1).

**Histological findings:** Pathological score significantly increased in the hearts of rats exposed to WTS in comparison with the CTL and Ex groups (P < 0.01). The combination of exercise and WTS attenuated the severity of lesions (P < 0.05 vs. S group); however, the pathological score was higher than that in the CTL group (P < 0.05) (Table 2) (Figure 4).

### Discussion

The present study was conducted to investigate the impact of the combination of WTS and swimming exercise training on heart electrical and mechanical performance.

Increase in plasma level of cotinine in animals exposed to WTS confirmed the smoking protocol



**Figure 4.** Hematoxylin and eosin (H&E) stained sections of heart tissue in different animal groups [a, CTL group heart sections showing normal appearance of cardiac myofibers; b, Ex group; c, S group; d, S + Ex group]

(→): Cellular hypertrophy; (...>): Congestion; (⇨): Interstitial edema; (➡): Cellular degeneration along with inflammatory processes

The magnification is × 40.

**Table 2.** Heart histopathological scores and the number animals with different degrees of injury in each group

Groups	Pathological scores of hearts					Mean
	No change	Minimum	Mild	Moderate	Sever	
CTL	4	1	0	0	0	0.2
S	0	0	0	1	5	3.83 <sup>#</sup>
Ex	2	2	1	0	0	0.8
S+ Ex	0	2	1	1	1	2.2 <sup>*¥</sup>

n = 5-6; CTL: Control; S: Smoking; Ex: Exercise; S + Ex: Smoking + Exercise; #P < 0.01 vs. CTL and Ex groups; ¥P < 0.05 vs. S group; \*P < 0.05 vs. CTL and Ex groups

0 = No change; 1 = Minimum (slight congestion and interstitial edema); 2 = Mild (small congestion and interstitial edema); 3 = Moderate (diffuse congestion and hypercontraction band, and/or with slight degree of leukocyte infiltration); 4 = Severe (hypereosinophilia, diffuse inflammatory process, and/or myofibrillar degeneration)

accuracy. There was an increasing trend in electrocardiogram (ECG) intervals following swimming exercise and WTS, but this was not significant. There was no significant difference in ECG and heart rate variability (HRV) parameters in the experimental groups. WTS induced negative effects on heart mechanical function including reduction in  $-dp/dt$  Max,  $+dp/dt$  Max, and contractility index and increase in the LVEDP and Tau index of the heart. It also induced pathological changes in the hearts of the animals.

Our finding indicated that swimming exercise training significantly attenuated pathological changes, improved the antioxidant system, modulated the pro-/anti-inflammatory systems, and effectively eliminated the negative effects of WTS on cardiac function.

Many human studies have investigated the acute effects of WTS on BP and HR. Cobb et al. compared a single acute exposure to tobacco-based and tobacco-free waterpipe products (40 minutes), and found only tobacco exposure to be associated with an increase in BP and HR.<sup>12</sup> Previous studies reported that HR and BP showed an increase immediately after each session of WTS exposure; however, we could not find any human study regarding the chronic effect of WTS on BP and HR. Nemmar et al., in an animal study, reported that chronic WTS increased BP in mice.<sup>13</sup> The discrepancy between our results and human studies can stem from the time interval between the end of WTS sessions and measurement of HR and BP parameters. All of the mentioned human studies recorded the hemodynamic parameters immediately or several minutes after each WTS; however, we measured the HR and BP of the animals 24 hours after the final smoking session. The hemodynamic alteration following each WTS session may be transient and this side effect can subside after a few minutes or hours. In comparison with a cigarette, 45 minutes of hookah smoking causes a threefold and twofold increase in nicotine level and the amount of carbon monoxide in the blood, respectively.<sup>14</sup> Because of the high affinity of hemoglobin for carbon monoxide (more than 240 times that for oxygen), hookah induced temporary cardiac hypoxia, which in turn triggers a rise in heart rate and heart workload.<sup>15</sup> To our knowledge, there is no human study which has chronically investigated the effect of WTS on hemodynamic

parameters. The studied species and measurement methods of parameters are other factors that can affect the results. Nemmar et al. measured the BP of conscious mice using mouse tail cuff. Due to being an indirect measurement, the animal being aware of the measurement, and being stressful and unbearable for the animal, the cuff method is usually associated with error. However, through the cannulation of the carotid artery in anesthetized animals, we measured the parameters directly and precisely. In addition, the difference in the brand of hookah tobacco, duration of each smoking session, and total duration of the sessions are others reasons for discrepancy between the results of the present study and the studies by Nemmar et al.<sup>13,16</sup>

The negative effect of WTS on systolic and diastolic heart mechanical function (decreasing contractility indices and increasing relaxation time indices) is an important finding of this study. In agreement with our findings, Dogan et al. showed that acute smoking changed the diastolic left ventricular function; it decreased transmitral E/A ratio and early diastolic velocity.<sup>17</sup> Another study demonstrated that WTS-induced left ventricular diastolic dysfunction is dose dependent.<sup>18</sup> A cross-sectional analysis among 50,045 residents of Golestan, Iran, showed a significant association between heavy waterpipe smoking and heart disease.<sup>19</sup> Furthermore, a population-based study showed association between WTS and traditional cardiovascular risk factors such as obesity, metabolic syndrome, diabetes, and dyslipidemia.<sup>20</sup> Moreover, tobacco smoke-induced systolic dysfunction (reduction in  $+dp/dt$ ) is reported in an animal study by Minicucci et al.<sup>21</sup> The harmful effects of WTS may result from the effect of the toxic components of tobacco smoke. In comparison with cigarette smoke, waterpipe tobacco smoke contains 6.5 times more CO, 1.7 times more nicotine, and 46 times more tar.<sup>22</sup> In addition, waterpipe smoke contains toxicant metals such as arsenic, beryllium, chromium, cobalt, lead, and nickel, which originate from the coal used in the waterpipe.<sup>15</sup> Charcoal combustion released a mainstream hookah smoke that is highly enriched with PM 0.1 (mean particle size of 0.04  $\mu\text{m}$ ), which is postulated to be more toxic than PM 2.5 present in cigarettes.<sup>23</sup> These toxic agents can cause inflammation and redox system imbalance

in favor of overcoming of oxidant factors that in turn can damage the heart.<sup>24</sup> Moreover, a single session of waterpipe smoking releases a larger quantity of smoke than a single cigarette, and the smoker inhales it with more force due to long path (hose) and its crossing the water chamber.

As mentioned above, the combination of mild exercise training and WTS prevented the negative effect of WTS on the heart. Park et al. showed that aerobic exercise mitigates the effects of cigarette smoking on arterial stiffness in young men, which is consistent with the results of the present study.<sup>25</sup> Furthermore, an animal study reported that the combination of exercise training with tobacco smoking prevents fibrosis, but has adverse effect on myocardial mechanics.<sup>26</sup> This study assessed the mechanical function of the papillary muscle in vitro by use of papillary muscles; however, we measured the mechanical function of the heart in vivo that can be the reason for the discrepancy between the results. Physical exercise-induced physiological cardiac hypertrophy and renewal can elevate cardiac function and prevent CVD.<sup>27</sup> Endurance swimming exercise, through diminishing the expression of the transcription factor C/EBP $\beta$  and increasing the expression of ED-rich carboxy-terminal domain 4 (CITED4), induces cardiomyocyte proliferation and hypertrophy in mice.<sup>28</sup> In addition, exercise training implements its cardiovascular protective effects through the up-regulation of anti-oxidative defense, promotion of angiogenesis and vascular endothelial function, enhancement of mitochondrial biogenesis, and reduction of inflammation and apoptosis.<sup>27</sup> We found that the level of pro-inflammatory ILs, such as TNF- $\alpha$ , IL-1 $\beta$ , and IL-6, increased in animals exposed to WTS; this increase was significant in TNF- $\alpha$ . This finding is consistent with results of another study

which indicated that TNF- $\alpha$  and IL-6 concentration increased in the heart of mice exposed to nose-only WTS.<sup>16</sup> It has been acknowledged that smoking causes oxidative stress,<sup>9</sup> and oxidative stress in turn causes cellular and tissue damage. The oxidant/antioxidant imbalance, through increasing pro-inflammatory cytokines production, probably induces pathological changes in the heart and results in its dysfunction. Our data indicated that exercise increases the activity of catalase and SOD, two important antioxidant enzymes, in the heart of animals exposed to WTS. Therefore, exercise trainings likely alleviated pathological changes and cardiac dysfunction induced by WTS partly through balancing the oxidant/antioxidant system and decreasing the level of pro-inflammatory ILs.

## Conclusion

The findings revealed that chronic WTS induces cardiac lesions and left ventricular systolic and diastolic dysfunction, and the combination of swimming exercise training with WTS prevents these negative effects. The beneficial effects of exercise can stem from its improvement of the antioxidant system and attenuation of the level of pro-inflammatory ILs. Understanding the precise mechanisms behind this effect requires further studies.

## Conflict of Interests

The Authors have no conflict of interest.

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## تأثیر آموزش تمرین استقامتی بر اختلال عملکرد قلب ناشی از مصرف دود قلیان

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### مقاله پژوهشی

### چکیده

**مقدمه:** در سراسر جهان، افزایش محبوبیت مصرف دود قلیان (Waterpipe tobacco smoking یا WTS) در بین جوانان و حتی در ورزشکاران وجود دارد. با وجود شواهدی مبنی بر اثرات مضر دود قلیان بر سیستم‌های مختلف بدن به ویژه سیستم قلب و عروق، اثر هم‌زمان آن با تمرینات ورزشی به خوبی مطالعه نشده است. هدف از انجام پژوهش حاضر، بررسی اثرات مواجهه WTS با/ بدون ورزش شنا بر فشار خون، بافت‌شناسی و عملکرد مکانیکی قلب موش‌های نر بود.

**روش‌ها:** نمونه‌های حیوانی به چهار گروه شاهد (CTL)، مصرف‌کننده دود قلیان (S)، ورزش استقامتی سبک (Ex) و گروه مصرف‌کننده دود قلیان همراه با ورزش (Ex + S) تقسیم شدند. مدت زمان ورزش و مصرف دود ۸ هفته بود. در نهایت، شاخص‌ها ثبت و اندازه‌گیری گردید.

**یافته‌ها:** فشار خون و ضربان قلب اختلاف معنی‌داری را در گروه‌ها نشان نداد. WTS، میزان Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) قلب ( $P < 0/05$  در مقایسه با گروه شاهد) و ضایعات بافت قلبی ( $P < 0/05$  در مقایسه با گروه شاهد) و شاخص‌های  $+dp/dt \max$  و  $-dp/dt \max$  و انقباض‌پذیری قلبی را افزایش داد ( $P < 0/01$ ،  $P < 0/05$  و  $P < 0/05$  به ترتیب در مقایسه با گروه‌های شاهد و ورزش). شاخص Tau بطن چپ نیز در گروه WTS افزایش یافت ( $P < 0/05$  در مقایسه با گروه شاهد و  $P < 0/01$  در مقایسه با گروه‌های ورزش). با این حال، ترکیبی از ورزش و WTS سطح TNF- $\alpha$  را کاهش داد، فعالیت سوپراکسیداز دیسموتاز (Superoxide dismutase یا SOD) و آنزیم کاتالاز قلب را بهبود بخشید و مانع از تأثیر منفی دود قلیان بر عملکرد قلب و مورفولوژی آن شد.

**نتیجه‌گیری:** ورزش از طریق بهبود آنتی‌اکسیدان‌ها و تضعیف سیتوکین‌های ضد التهابی، مانع از اختلال عملکرد سیستولی و دیاستولی بطن چپ ناشی از WTS می‌شود.

**واژگان کلیدی:** قلیان کشیدن، تمرینات ورزشی، عملکرد بطن چپ، آنتی‌اکسیدان‌ها، سیتوکین‌ها

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