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Experimental Research Article

Long-term exposure to waterpipe tobacco smoke attenuates the positive effect of moderate exercise training on heart rate variability in male rats

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ABSTRACT

Introduction: According to earlier research, fitness training can reduce the cardiovascular risks of waterpipe tobacco use. This study aimed to investigate how electrocardiogram (ECG) parameters and heart rate variability (HRV) changes occur in Wistar rats exposed to continuous hookah smoke and moderate endurance exercise training.

Methods: 28 adult male Wistar rats, weighing 180–220 g, were divided into 4 groups as control group (CTL), exercise training group (Ex), waterpipe tobacco smoke exposure group (WPS), andexercise training and waterpipe tobacco smoke group (WPS+Ex). After completing the 8-week smoke exposure and exercise protocol, ECG recording, HRV characteristics, including frequency domains, temporal domains, and non-linear analysis, were computed.

Results: The findings showed that, compared to the control group, hookah smoking significantly increased the RR interval ($P < 0.05$) and decreased heart rate (HR) and standard deviation of the second heart rate variation (SD2), an indicator of long-term HRV variability. Compared to the CTL group, exercise training decreased the low frequency/high frequency (LF/HF) band non-significantly and increased the HF band while decreasing the LF band ($P<0.05$). Following exposure to hookah smoking and exercise training, the WPS+Ex group's SD2 rose ($P<0.05$), whereas the WPS group's HF and corrected QT (QTc) interval decreased.

Conclusion: Overall, our results demonstrated that 8 weeks of moderate-intensity endurance training had a slight favorable impact on the rats' HRV frequency domains. At the same time, hookah smoking inhibited this beneficial effect and lowered HR.

Keywords:
Tobacco smoking
Endurance training
Heart rate variability
Electrocardiogram
Animal model

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Introduction

Tobacco smoking is one of the most critical and serious health challenges in the world, which is spreading all over the world, especially among young people (Organization 2025).

There has been an increase in waterpipe smoking and a shift from cigarettes to waterpipe smoking in young people in recent years (Najafipour et al., 2022).

Hookah is one of the common forms of tobacco consumption. During the utilization of hookah, the smoke produced by heating the tobacco first passes through a chamber containing water before inhalation.

The possibility of removing toxins from hookah smoke due to the passage of smoke through water has been rejected (Darawshy et al., 2021), and hookah smoke includes harmful levels of toxins such as nicotine, polycyclic aromatic hydrocarbons, and heavy metals (Greige-Gerges et al., 2023).

A significant relationship between hookah consumption and respiratory and cardiovascular disorders, such as hypertension, coronary diseases, and heart failure, has been shown in human and animal studies (Alavi et al., 2021; Bhatnagar et al., 2019; Mahmoud et al., 2023; Nakhaee et al., 2020).

The physiological and measurable variation in the intervals between heartbeats is known as heart rate variability, or HRV. It is a measure of the heart's autonomic function, and a drop in it denotes either a decrease in parasympathetic activity or an excess of adrenergic activity. A reduction in HRV levels has been linked to an increased risk of death because it is one of the indicators determining the prognosis of heart disease. (Bashiri et al., 2023; Chang et al., 2020; Sen and McGill 2018). Human studies reported that short-term hookah smoking decreased HRV in hookah smokers (Al-Kubati et al., 2006; Cobb et al., 2012; Nelson et al., 2016) and this reduction in HRV was prevented by β-adrenergic blockade (Nelson et al., 2016). Earlier investigations show that exercise training improves HRV and, in turn, reduces the risk of heart disease (Deus et al., 2019; Raffin et al., 2019). Furthermore, exercise training can mitigate the adverse effects of hookah smoke on the body (Alavi et al., 2022). The combined impact of training and hookah smoking on HRV has not been well investigated in human and experimental studies; therefore, this study was designed to assess the long-term effects of hookah smoking with/without moderate endurance exercise on cardiac autonomic function by measuring HRV and whether the combination of these two interventions overshadows the effects of each alone on ECG and HRV.

Materials and Methods

Animals, Housing, and Study design

National criteria for the care and use of laboratory animals were followed in the conduct of this experiment, as approved by the Ethics Committee of Kerman University of Medical Sciences, Kerman (IR.KMU.REC.1397.541). The Al Fakher Company of the United Arab Emirates produced the Double Apple tobacco, which was bought from the marketplace.

Twenty-eight adult male Wistar rats weighing 180–220 g were purchased from the Kerman University of Medical Sciences animal farm. They were housed at 23 ± 2°C with a 12-hour light and 12-hour dark cycle. The rats were allocated into four groups of seven at random: the control group (CTL), the exercise group (Ex), which trained on a treadmill for eight weeks, the hookah smoking group (WPS), which was exposed to hookah smoke for eight weeks, and the exercise with hookah smoking (WPS+Ex) group, which was both exposed to hookah smoke and under training. The rats were anesthetized with an intraperitoneal injection of 50 miligram per kilogram sodium thiopental 48 hours following the conclusion of the final day of smoking and exercise. Blood pressure and Lead II of the ECG were recorded (Joukar and Sheibani 2017). Finally, at the end of the procedure, the rats were sacrificed with intraperitoneal administration of a euthanizing dose of ketamine (100 miligram per kilogram) and xylazine (80 milligrams per kilogram).

Moderate Intensity Exercise Training Protocol

For eight weeks, the animals were trained five days a week at a 0◦ slope on a treadmill. Exercise duration and intensity were progressively increased. Running on the treadmill was done at a pace of 8 meters per minute for 10 minutes in the first week, 12 meters per minute for 25 minutes in the second, 18 meters per minute for 40 minutes in the third, and 24 meters per minute for 60 minutes in the fourth and eighth weeks (Iwamoto et al., 1999). At 8 a.m., the animals were exercise trained in accordance with the previously mentioned routine. Seldom was a manual shock mechanism employed to

TABLE 1: The training protocol details.

Weeks	Speed (m/min)	Duration (min)
1th week	8	10
2th week	12	25
3th week	18	40
4-8th weeks	24	60

maintain the animals' motion on the treadmill (Table 1).

Waterpipe Tobacco Smoking Protocol

Animals in the smoking subgroups were exposed to waterpipe smoke five days a week for eight weeks, two hours after the diurnal activity training phase. The specifics of the smoking apparatus employed in this investigation were previously described. It was created and produced in our research laboratory (Nakhaee et al., 2019). To create conditions similar to Waterpipe smoking (WPS), a 50x30x15 glass chamber was used for the animals exposed to the smoke. The amount of tobacco consumed was 10 grams for30-minute periods of smoking. First, smoke from burning tobacco was pumped into the animal's chamber for 30 seconds. Then, fresh air entered the chamber for 30 seconds to remove the smoke. Afterward, the animals breathed fresh air for 30 seconds, and the next cycle was repeated. Every day, 20 cycles (20 × 1.5-min exposure = 30-min exposure session) were performed. To replicate the stress of the protocol environment, the CTL group's animals spent the same length of time in the chamber as the test groups. The carbon monoxide (CO) concentration was recorded using the Testo 310 (Germany) CO measurement device and was kept constant throughout all smoke inhalation sessions [mean ± standard deviation (SD): 918 ± 126 parts per million (ppm) (Alavi et al., 2021). Double Apple tobacco is widely consumed in Iran and is a well-known brand globally. Schubert et al. previously measured the average aromatic compounds of flavored waterpipe tobaccos and found that the Al Fakher Double Apple tobacco had roughly 500 mg/g of volatile flavor substances, such as ethyl 2-methyl butyrate, hexanal, limonene, 1-hexanol, cis-3-hexen-1-ol, benzaldehyde, linalool, L-menthol, benzyl acetate, trans-anethole, and benzyl alcohol (Schubert et al., 2013).

Blood sampling, Blood pressure, and ECG recording and HRV measuring

The animals were anesthetized 48 hours following the last training session by administering sodium thiopental (50 milligrams per kilogram) intraperitoneally. A 6-minute ECG recording was used to follow rat stability and anesthetic induction. A PowerLab bio-amplifier and a PowerLab physiograph recording device (AD Instruments, Australia) were used to record the Lead II ECG. The neutral electrode is attached to the right foot, the positive electrode to the left foot, and the negative electrode to the right hand for this reason. A cannula was then placed into the left carotid artery to take a blood pressure reading once it had been exposed. A pressure transducer and the PowerLab equipment were used to take blood pressure 15 minutes following the stabilization phase, which ended after surgery. Thereafter, blood sampling was done and serum was separated, and the cotinine level was measured by an ELISA kit according to its manufacturer's instructions. Figure 1 displays the blood pressure and ECG trace of an animal (WPS group) exposed to hookah smoke. The formula below was used to determine the mean arterial pressure (MAP):

MAP= Diastolic pressure + 1/3 pulse pressure (Botelho et al., 2019; Joukar and Sheibani 2017). ECG parameters and HRV components were measured by an ECG analyzer and HRV analyzer software, respectively.

In addition to the heart rate (HR) index (number of heartbeats per minute), other parameters of ECG were measured, including RR interval (the time interval between two R waves in two successive beats and has an inverse relationship with the HR), PR interval (from the beginning of the P wave to the start of the QRS complex), QTc (which is calculated using the formula $QTc=QT/\sqrt{RR}$ and helps that the RR interval does not affect the interpretation of the QT interval). The following parameters were also computed using HRV software: Average SDRR (standard deviation of regular RR interval) and RMSSD (second root of the mean square difference of normal RR distance), average LF (frequency band 0.2-0.75 in spectral analysis, a non-spe-

FIGURE 1. The Strips of Arterial Blood Pressure and Lead II of ECG Recorded From an Animal of WPS Group

cific indicator of the influence of the two parts of the autonomic system), average HF (frequency band 0.75-2.5 in spectral analysis, an indicator of parasympathetic activity), average LF/HF (low to high-frequency spectral band ratio, an indicator of autonomic system balance), SD1 (vertical standard deviation compared to the identity line in the Poincare plot), SD2 (standard deviation along the identity line), SD1/SD2 (the ratio of the vertical standard deviation to the alignment in the Poincaré plot).

Statistical analysis

The mean ± standard error of the mean (SEM) was used to express the results. SPSS software version 20 (Inc., Chicago, IL, United States) was used to conduct the statistical tests, and a significant level ($P<0.05$) was taken into account. The Shapiro-Wilk test was used to evaluate the normalcy hypothesis. Statistical comparisons were conducted using One-way ANOVA and Tukey post-hoc tests.

Results

Cotinine

Serum cotinine level as a valid marker of smoking status was significantly increased in the WPS group compared to the CTL group (8.56 ± 0.52 vs. 22.20 ± 1.08, $P<0.001$). In comparison with WPS, smoking along with exercise training group showed a lower level of serum cotinine (18.44 ± 0.85 vs. 22.20 ± 1.08, $P<0.01$) but still was higher in comparison with non-smoking groups ($P < 0.001$).

ECG results

According to our findings, the HR of the WPS group reduced significantly after eight weeks when compared to the CTL group ($P<0.05$), but this parameter did not show significant changes in the other studied groups (Fig. 2A). The mean RR Interval was substantially larger in the hookah-smoked animals than in the CTL group ($P<0.05$), but there was no remarkable difference in the other groups (Figure 2B). There was no discernible difference in the mean PR Interval between the experimental groups (Figure 2C). There was no discernible difference between the other groups, and the exercise group with hookah smoke consumption (WPS+Ex) had a significantly shorter QTc interval than the exercise group (Ex) ($P<0.05$) (Figure 2D).

HRV indices

The effects of waterpipe smoking and exercise training on the time domain parameters of HRV, RMSSD (Figure 3A), and SDRR (Figure 3B) were not statistically significant in this investigation. On the other hand, exercise training by itself considerably raised the HF

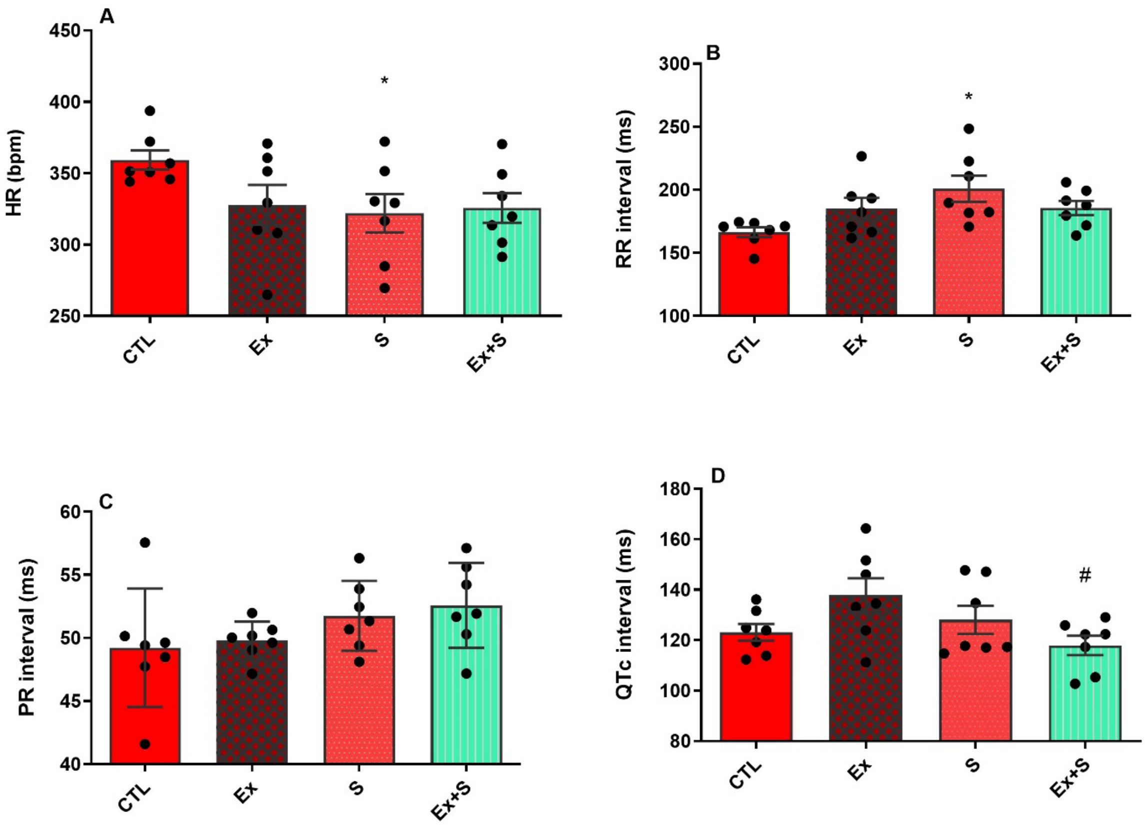

FIGURE 2. Effects of waterpipe smoke inhalation on HR, HR (A); RR, the time between successive R-waves in ECG (B); PR, the time from the onset of the P wave to the start of the QRS complex in ECG (C); QTc, corrected QT interval as Bazett's formula normalized (D). Values are expressed as mean±SEM. n (7); CTL, control group; Ex, group which was subjected to exercise training for 8 weeks; WPS group, which was subjected to waterpipe tobacco smoke inhalation for 8 weeks; WPS+Ex, group which was subjected to exercise training and waterpipe tobacco smoke inhalation for 8 weeks. *p<0.05 compared with CTL, #p<0.05 compared with Ex.

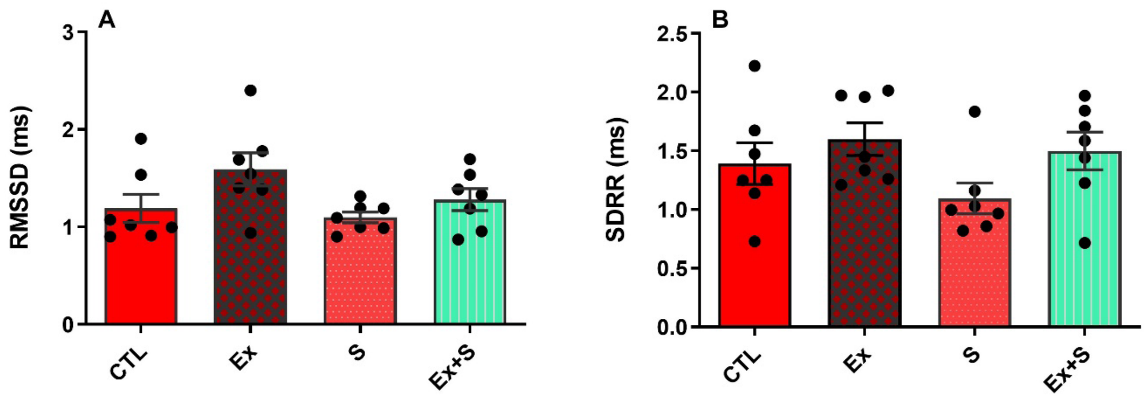

FIGURE 3. Effects of waterpipe smoke inhalation on RMSSD, a time domain parameter of HRV: square root of the mean squared differences of successive RR intervals (A); SDRR, standard deviation of RR intervals (B). Values are mean±SEM, n (7); CTL, control group; Ex, group which was subjected to exercise training for 8 weeks; WPS, group which was subjected to waterpipe tobacco smoke inhalation for 8 weeks; WPS+Ex, group which was subjected to exercise training and waterpipe tobacco smoke inhalation for 8 weeks.

parameters and decreased the LF (P<0.05). When compared to the exercise group (Ex), the WPS group's mean low frequency (LF) increased significantly (P<0.05).

Nevertheless, there was no discernible connection between the other groups (Figure 4A). In comparison to the Ex-group, smoke inhalation significantly decreased the

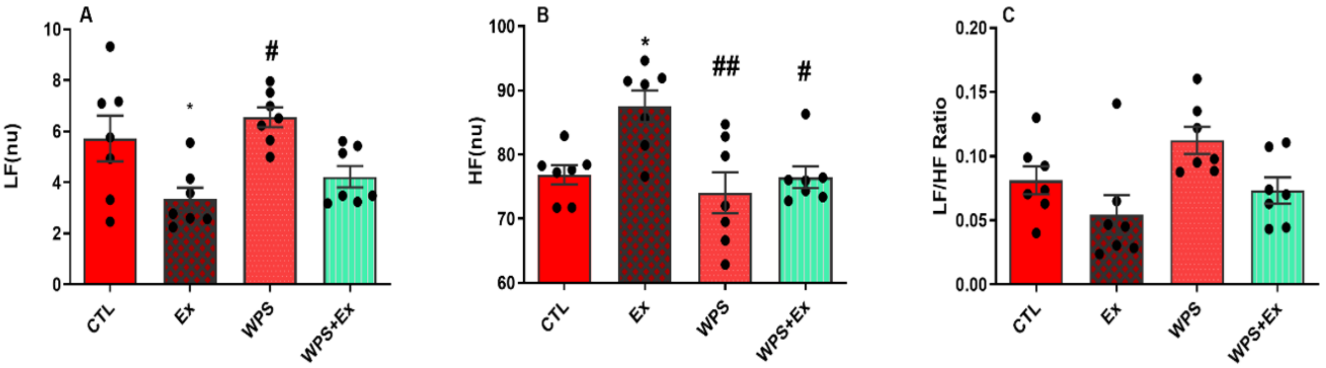

FIGURE 4. Effects of waterpipe smoke inhalation on LF, low frequency (A); HF, high frequency (B), two parameters of frequency domain (spectral) of HRV, and LF/HF ratio (C). Values are mean±SEM, n (7); CTL, control group; Ex, group which was subjected to exercise training for 8 weeks; WPS, group which was subjected to waterpipe tobacco smoke inhalation for 8 weeks; WPS+Ex, group which was subjected to exercise training and waterpipe tobacco smoke inhalation for 8 weeks. *p<0.05 compared with CTL, #p<0.05, ##p<0.01 compared with Ex.

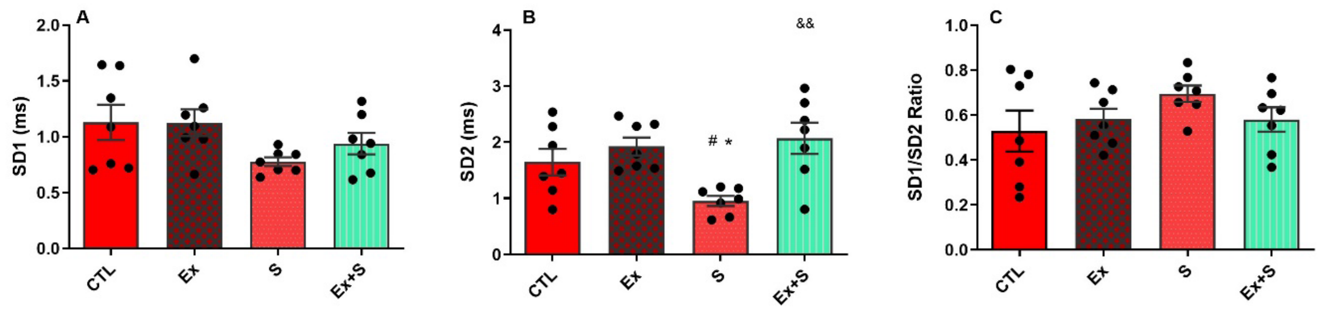

FIGURE 5. Effects of waterpipe smoke inhalation on SD1 (A); SD2 (B), the standard deviation of the Poincar′e plot perpendicular to (SD1) and along (SD2) the line of identity, and SD1/SD2 ratio (C). Values are mean±SEM, n (7); CTL, control group; Ex, group which was subjected to exercise training for 8 weeks; WPS, group which was subjected to waterpipe tobacco smoke inhalation for 8 weeks; WPS+Ex, group which was subjected to exercise training and waterpipe tobacco smoke inhalation for 8 weeks. *p<0.05 compared with CTL, #p<0.05 compared with Ex, &&p<0.01 compared with WPS. SD1 is an index of short-term variability, and SD2 is an index of long-term variability.

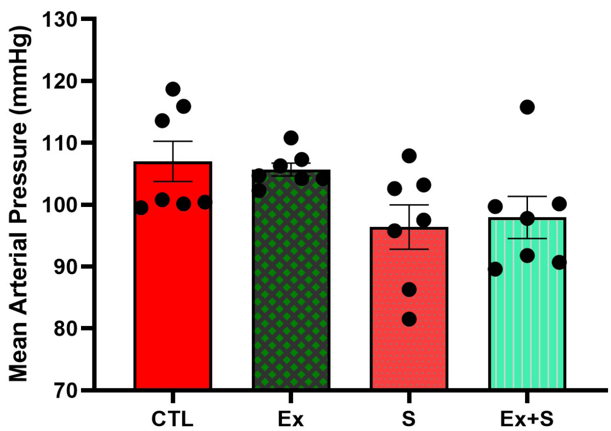

FIGURE 6. Effects of waterpipe smoke inhalation and moderate-intensity exercise training on MAP. Values are mean ± SEM, n (7); CTL, control group; Ex, group which was subjected to exercise training for 8 weeks; S, group which was subjected to waterpipe tobacco smoke inhalation for 8 weeks; Ex + S, group which was subjected to exercise training and waterpipe tobacco smoke inhalation for 8 weeks.

HF values in the WPS (P<0.01) and WPS+Ex (P<0.05) groups (Figure 4B). Nonetheless, there was no discernible difference in the LF/HF ratio between the groups under study (Figure 4C).

According to data analysis, the SD1 parameter was not significantly impacted by smoking, exercise, or both (Figure 5A). Exercise combined with smoke inhalation reversed the effects of hookah smoking (P<0.01), and 8 weeks of smoking significantly reduced SD2 values in the hookah smoking group when compared to the CTL and Ex groups (P<0.05) (Figure 5B). There was no remarkable difference in the SD1/SD2 ratio results between the groups (Figure 5C).

Mean arterial pressure (MAP)

Data analysis showed that smoking, exercise, and their combination had no remarkable impact on the MAP (Figure 6).

Discussion

The primary objective of this study was to determine the effect of long-term exposure to waterpipe tobacco smoke and moderate exercise training on ECG parameters and HRV indices in male rats and whether the combination of these two interventions overshadows the effects of each alone on ECG and HRV.

The findings demonstrated that while moderate-intensity exercise improved several HRV measures in rats, hookah use negated these benefits. Furthermore, hookah smoke by itself was linked to a notable drop in HR but had no discernible impact on blood pressure.

According to a systematic review by Chaieb et al., smoking a hookah is linked to a lower cardiovascular response to exercise and a lower submaximal and maximal aerobic capacity. (Chaieb and Ben Saad 2021). Additionally, it may be linked to aberrant cardiovascular status, such as elevated blood pressure and HR at rest, lowered HR and propensity for chronotropic failure after exercise, and a lower recovery index during the recovery phase (Chaieb and Ben Saad 2021).Because the length, frequency, and quantity of hookah smoke inhalation varied between this investigation and the Chaieb et al. review, the results of the effect of hookah smoke on HR differed. Nicotine from smoking is thought to enhance the production of norepinephrine by stimulating nicotinic acetylcholine receptors in the sympathetic ganglia. This, in turn, is thought to raise HR by stimulating adrenergic beta receptors (Chaieb and Ben Saad 2021). Our findings are in line with research showing that chronic smoking lowers resting HR, which may be caused by beta-adrenergic receptors becoming desensitized or downregulated as a result of the body's prolonged release of norepinephrine after high nicotine levels (Iaccarino et al., 2005; Papathanasiou et al., 2013), anyway, confirms the adverse effects of hookah on heart activity.

Exercise lessened the negative effects of smoking on the shape and function of the heart. The results of our most recent animal study demonstrated the effects of hookah smoke exposure with and without swimming on blood pressure, cardiac histology, and mechanical activity in male Wistar rats. Furthermore, the blood pressure of normotensive rats that received exercise training, waterpipe tobacco smoking, or both did not significantly change as a result of these interventions, which is consistent with this investigation (Nakhaee et al., 2019). The combination of sympathetic and parasympathetic activity is linked to the long-term variability expressed by the LF (ms2), SDRR, and SD2 indices. On the other hand, HF (ms2), RMSSD, and SD1 are measures of vagal tone-induced short-term variability. The sympathovagal balance is indicated by the ratio of LF (n.u)-HF (n.u) (LF/HF) (Chen et al., 2015; Joukar and Ghorbani-Shahrbabaki 2016).

The lack of statistically significant differences in RMSSD and LF/HF may be attributed to the relatively small sample size and the high sensitivity of these indices to physiological conditions in conscious and anesthetized animals. Future studies with larger sample sizes could provide a clearer understanding of the effects of exercise and hookah smoke on HRV parameters.

By decreasing LF and increasing HF, moderate-intensity exercise training enhanced HRV frequency domains in this investigation. The reduction impacts of hookah smoking on SD1/SD2, another measure of sympathovagal balance, did not reach a meaningful level, however, as it considerably decreased SD2 and non-significantly dropped the SD1 parameters. It's interesting to note that waterpipe tobacco smoking reduces the beneficial impacts of training on HRV frequency domains in the animal group that received both hookah and exercise.

Prolonged inhalation of hookah can cause a decrease in vagal nerve activity or a relative increase in sympathetic cardiac activity at rest and after exercise. This resulted in a higher HR, a lower standard deviation of

RR intervals, and a decrease in total HRV parameters, according to an analysis of a Wingate test. (Ahmadian et al., 2018).The variation in the type of exercise and the time between the last hookah session and the time of the ECG and HRV recording may be the cause of the divergence between their findings and our study's findings. An ECG taken right after smoking cigarettes is said to show a drop in parasympathetic activity (Talukdar et al., 2020). HRV, a measure of resting cardiac autonomic nerve function, was affected by long-term hookah smoking, and the post-Wingate cardiac recovery (HRR) was comparable to that of non-smokers (Ahmadian et al., 2018).

According to a human study, acute waterpipe smoking causes the myocardium to consume more oxygen while lowering HRV. This is an indirect sign of adrenergic hyperactivity, and propranolol's beta-adrenergic suppression reverses this effect (Nelson et al., 2016). The contrast with the current study is justified by the fact that this decrease in HRV was obtained by comparing the values before and immediately after ingestion. The detrimental effects of nicotine and tobacco's fine particles on the autonomic nervous system's operation were examined, with a focus on the processes behind both acute and long-term sympathetic nervous system regulation(Middlekauff et al., 2014). Reactive oxidative species and sympathetic nerve activity maintain it through a positive feedback loop. Therefore, the considerable rise in LF and decrease in HF in the hookah group relative to the exercise group can be confirmed by the increase in sympathetic activity that occurs after waterpipe smoking.

There have been prior reports of the benefits of exercise modalities on HRV, particularly strength training and high-intensity interval training (Caruso et al., 2015; Holmes et al., 2022; Yang et al., 2024). However, investigations revealed that there is little to no impact of moderate endurance training on sympathovagal balance and HRV (Cornelissen et al., 2010; Lundstrom et al., 2023), which confirms our findings. It seems that duration, modality, and intensity determine the impacts of training on HRV (Lundstrom et al., 2023). Future research is necessary because the variation in HRV balance response to various exercise modalities and intensities may be related to the vagal critical point, which establishes the responsiveness threshold.

As previously stated, research on humans has demonstrated that after prolonged endurance exercise, resting HR drops by 4-9% (Reimers et al., 2018). Considering the huge sample size in human research, this degree of HR reduction is noteworthy. The sample size is typically small in animal research like this one, and the HR decrease brought on by moderate-intensity endurance exercise could not be statistically significant (Baravati et al., 2015). The question that has to be addressed here is whether sensitivity or beta-adrenergic receptor count decreases or other mechanisms are to blame for the HR drop that occurs after endurance exercise, which is comparable to smoking. According to a human study, exercise training does not cause a drop in beta 1-adrenergic receptor responsiveness or an increase in parasympathetic tone, which would explain the decrease in resting HR. Based on the authors, a decrease in HR during exercise is probably caused by a decrease in intrinsic HR (Bahrainy et al., 2016). An animal investigation showed that training-induced bradycardia endures following inhibition of the autonomous nervous system in vitro in the denervated sinus node and in vivo in mice, supporting the aforementioned human study. They revealed changes in the ion channels of pacemakers, specifically a downregulation of the If ionic current and HCN4. Additionally, they found that the downregulation of HCN4 is explained by the overexpression of transcriptional regulators miR-1 and NRSF, and the downregulation of Tbx3 brought on by exercise training (D'Souza et al., 2014). Thus, based on what is currently understood, the bradycardic impact brought on by prolonged hookah use cannot be explained by a decrease in the quantity or sensitivity of beta-1 receptors in the heart, which is one explanation for the drop in resting HR following endurance exercise. The mechanism by which exercise and hookah smoke lower HRs may differ, which could explain why they have opposing effects on HRV. Further research is needed to confirm this.

The comparison between the exercise-only and hookah-smoking–only groups provides insight into the opposing influences of regular physical activity and waterpipe smoke exposure on HRV. This contrast highlights the extent to which exercise-driven improvements in autonomic balance mayor may notoffset the negative cardiovascular consequences of waterpipe tobacco smoke, offering clinically relevant information for populations that combine smoking habits with physical activity.

Limitation

The lack of addressing the mechanisms involved in the effect of exercise on the effects of smoking and vice versa on measured cardiac parameters, as well as how the expression of heart beta receptors and ion channels changes, are limitations of this study that will be addressed in future studies by our research team.

Conclusion

Overall, our findings revealed that eight weeks of moderate-intensity endurance training has a mild impact on improving the frequency domains of HRV in rats, and hookah smoking suppresses this positive effect. In addition, long-term waterpipe tobacco smoking may decrease resting HR. It is speculated that this effect is due to a downregulation of heart beta receptors due to long-term stimulation of nicotinic acetylcholine receptors in the sympathetic ganglion by nicotine in tobacco. These findings highlight the potential cardiovascular risks of waterpipe smoking for physically active individuals and support public health recommendations advocating cessation of waterpipe tobacco use, particularly among athletes. Future studies should explore dose-response relationships and longer exposure durations to provide a more detailed understanding of the effects of waterpipe tobacco smoke and exercise on HRV parameters.

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Conflict of interest

The authors declare there is no conflict of interest.

Ethics approval

The KUMS' Institutional Animal Care and Use Committee approved the procedures (Ethical code: IR.KMU.REC.1397.541).

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