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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Effects of cigarette smoking and l-nicotine on isolated trachea, aorta and perfused heart of albino rat

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Abstract

This work was an attempt to reveal the effects of a Jordanian cigarette smoke and pure L-nicotine on the contractile activity of tracheal and aortic smooth muscles, and isolated perfused heart of albino rat. In tissue bath experiments, cigarette smoke extracts (CSE) and L-nicotine induced biphasic change in the tone of isolated precontracted-tracheal and aortic smooth muscles. In the case of isolated perfused heart, CSE and L-nicotine induced in most concentrations a significant concentration dependent reduction of the force and the rate of contraction of the perfused heart. One gram of cigarette tobacco produces an equivalent of 4.8 mg L-nicotine in cigarette smoke extract.

Keywords: Cigarette smoke; L-nicotine, trachea; aorta; perfused heart; albino rat

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Introduction

Smoking is the act of inhaling and exhaling the fumes from burning plant materials, especially tobacco. It is consumed in the form of cigarettes, cigars, chew, pipes or water-pipe (Hoffmann and Wynder, 1986). About 1.25 billion humans smoke cigarettes daily and therefore smoking presents a worldwide social problem (Proctor, 2001).

The smoke generated from burning tobacco is divided into two types: mainstream smoke and sidestream smoke. The mainstream is the smoke which is inhaled by the smoker from tobacco product during puffing. The sidestream is the smoke which is emitted by burning cigarette between puffs. The sidestream smoke usually contains higher concentrations of toxic and carcinogenic agents than the mainstream smoke (Stephen, 2010).

Cigarettes contain more than 4,000 identified chemical compounds including 60 known carcinogens (Stephen, 2010). The gaseous components of mainstream smoke (92% of the total smoke) involve 400-500 different gases which include carbon-monoxide, nitrogen oxide, hydrogen cyanide, formaldehyde and ozone. Particulate matter (8% of mainstream smoke) contains tar products such as naphthalene, pyrene and nitrosamine (Stephen, 2010) and metals such as cadmium, polonium, selenium, mercury, lead and arsenic (Galażyn-Sidorczuk et al., 2008; Stephen, 2010). The deleterious effect of cigarette smoking on the cardiovascular system, would lead to coronary artery disease, atherosclerosis and peripheral vascular disease. Also, smoking has been implicated in the development of cerebrovascular diseases and aortic dilatation (Goich, 1995; Leone, 1995; Nakamura, 2008; Desai et al., 2009).

The carbon-monoxide and nicotine of tobacco smoking have been mainly implicated in acute cardiovascular disease (Thomas, 1993; Higman and Powell, 1994; Maziak, et al., 2004). The nicotine in cigarette stimulates the sympathetic nervous system with consequent effect on the heart rate and peripheral vasoconstriction with consequent elevation of blood pressure (Higman and Powell, 1994). The physiological effects of carbon monoxide are associated with increased carboxy-hemoglobin level,

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which reduces the oxygen capacity of blood (Higman and Powell, 1994). According to a report of Public Health Laboratories, Maryland, USA (1997), the Jordanian cigarettes contain about twice the amount of nicotine and tar which is found in non-Jordanian cigarettes. Hadidi and Mohammed (2004) determined the nicotine content of Jordanian tobacco used in hubble-bubble smoking. Their analysis showed an average of 8.32 mg/g tobacco (1.8-41.3 mg/g).

This study aims to investigate the effect of CSE and L-nicotine on isolated, perfused heart, aorta and trachea of albino rat.

Aims of the work

1. Determination of nicotine concentration in a selected Jordanian cigarette smoke extracts (CSE).
2. To evaluate the response of isolated precontracted-aortic and tracheal tissues to CSE and to pure L-nicotine, under isometric conditions.
3. To study the isometric contractions of isolated perfused heart treated with CSE and pure L-nicotine.

Materials and Methods

Male albino rats (*Rattus Norvegicus*) weighing 200–250 g were obtained from Department of Biological Science, University of Jordan.

Isolated, perfused heart system

Male rats (200–250 g) were pretreated with heparin (500 U/100 g) approximately 20 min before rat decapitation. Whole heart was carefully excised with the main trunk of the aorta remaining attached. In order to trim excess fat, the excised heart sample was transferred to a Petri-dish containing ice-cold buffered physiological saline solution (PSS) containing (mM): NaCl 118, KCl 4.7, $CaCl_2.2H_2O$ 2.5, $MgCl_2.6H_2O$ 0.5, $NaHPO_4$ 1.0, $NaHCO_3$ 25, D-Glucose 11.1. The aorta was tied onto a glass cannula connected to a reservoir located 70 cm above the heart. The reservoir contained PSS which was continuously aerated, with a mixture of 95% O_2 and 5% CO_2. The PSS temperature was maintained at 37°C. The perfusion was carried out at a constant flow rate of 5 to 7 ml/min.

A small light stainless steel hook was inserted into the heart apex and connected by a thread to a force transducer (FT-302, CB Sciences, USA). This was further connected to a pen recorder (Cole Parmer, Chicago, 1L). The isometric contractions of the beating heart were recorded under a tension of approximately 1 g. A syringe with needle attached to the side of the glass cannula was used to inject the test solution at specific concentrations immediately above the heart (Abdalla et al., 1992).

Preparation of cigarette smoke extracts (CSE)

CSE was prepared by bubbling mainstream smoke of one filter cigarette (containing 0.7 g tobacco) into 2 ml phosphate-buffered saline (0.1M/ pH 7.4) during 2.5 minutes (Murohara, 1994).

The effect of cigarette smoke extract and L-nicotine on the isolated perfused heart

An equilibration period of 20-30 min with fresh PSS perfusate was performed to stabilize the heart rate. Then, various concentrations of CSE and L-nicotine (beginning with the lowest concentration) were individually injected through a needle located immediately above the aorta. The heart was perfused with aerated, buffered PSS before the injection of the next compound concentration to ensure reaching a stable record. Changes in heart rate as well as in force of contraction were observed, and the results were expressed as a percentage value relative to the control value obtained immediately before the addition of the CSE or L-nicotine.

Tissue bath experiments

A slight anesthetization of the animal by inhaling diethyl ether was rapidly followed by decapitation. Then, either the aorta or trachea was dissected out and immediately transferred into a warm aerated buffered PSS (pH 7.4) before the clearing of adherent fat and connective tissue. One ring (2-3 mm in length) was obtained, and suspended in a water-jacketed tissue bath, filled with 9 ml fresh buffered PSS. The ring was horizontally oriented and suspended by two wire clips passed through the lumen; one clip was anchored to the bottom of the tissue bath, while the other was connected to a force transducer (FT-302, CB Sciences, USA) (Schramm, 2000). The tissue was then progressively stretched (approximately 1g for both aorta and trachea). The tissue bath solution was aerated with 5% CO_2 and 95% O_2 gas mixture, and the temperature was maintained at 37°C throughout the experiment. Rings of either aorta or trachea were then allowed to equilibrate for about 60 min and 90 minutes, respectively. To protect against interfering metabolites, buffer replacements were carried out every 15 minutes prior to further exposure to any test solution. Isometric tension was measured and recorded on a pen recorder (Cole Parmer, Chicago, 1L) (Schramm, 2000; Martin et al., 1997).

Concentration-effects of CSE and pure L-nicotine on the contractility of aortic and tracheal rings

After an equilibration period (as described above), the aortic rings were precontracted with 0.01 mM phenylephrine (PE). Then various concentrations of CSE (0.1, 0.3, 1, 3, 10, 30, 60 and100 µl/ml), or pure L-nicotine (2.4, 7, 24, 70, 240, 700, 2400 µg/ml), were

added in a cumulative manner to the tissue bath. This was followed by addition of the non-specific relaxant papaverine (Pap) at 1 mM to induce maximum relaxation. The magnitude of the response induced by each added aliquot was calculated and expressed as a percentage of the maximum response induced by papaverine. The higher concentration was not added until the tissue response to the previous concentration reached a plateau. Trachea rings were treated similarly by CSE (µl/ml), and pure L-nicotine (µg/ml), except that the initial pre-contraction was achieved by 0.02 mM carbachol (CCh) (Lotriet et al., 2007).

Spectrophotometric determination of L- nicotine in CSE

For colorimetric determination of L-nicotine concentration in CSE, the method described by Al-Tamrah (1999) was followed. This method is based on the development of a green colour due to the reduction of potassium permanganate to the manganate. The green colour indicates the oxidation of nicotine in the presence of sodium hydroxide. The green product absorbs strongly at wavelength of 610 nm (Al-Tamrah, 1999).

Protocol

The following procedure was adopted using a 25 ml volumetric flask:

1- Adding 1 ml of 0.0125 M potassium permanganate, followed by 2 ml of 6.25 M NaOH.

2- After a gentle mix, the desired amount of phosphate-buffered saline alone, phosphate-buffered saline with nicotine, or that of a CSE was added, and the volume was made up to about 20 ml with distilled water.

3- The mixture was then heated in a water bath at 100 °C for approximately 7.5 min.

4- The solution was cooled to room temperature, then, the volume was adjusted to 25 ml with distilled water.

5- Finally, the absorbance was measured at 610 nm against a reagent blank using a UV-9200 spectrophotometer (Al-Tamrah, 1999).

Results

Spectrophotometric determination of L-nicotine in CSE

One LM-red Jordanian cigarette contains 0.7 g tobacco. Using the spectrophotometric method described by Al-Tamrah (1999), a standard curve was prepaired for pure L-nicotine to find the nicotine concentration in CSE. Calculations showed that 1 g cigarette tobacco gives rise to an equivalent of 4.8 mg L-nicotine in CSE.

Tissue bath experiments:

Effect of cigarette smoke extract (CSE) on isolated smooth muscle preparations

CSE, in concentrations ranging from 0.1-100 µl/ml caused biphasic tension change in carbachol-precontracted trachea (P<0.05) (Fig. 1 & 5). Also, CSE in concentrations ranging from 0.1-100 µl/ml induced biphasic tension change in isolated rat aorta during stable contraction to phenylephrine (P< 0.05) (Fig. 2 & 5). The maximum contractile and relaxant effect of CSE on isolated aorta and trachea are shown in Table 1.

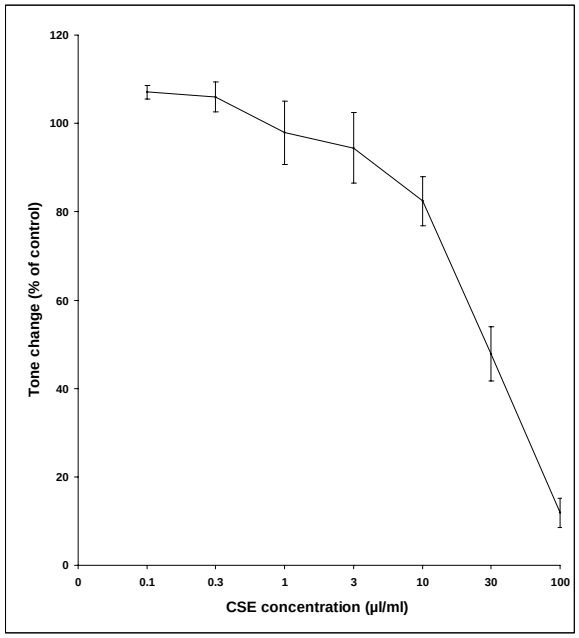

Fig. 1: Concentration-effect curve of CSE on carbachol-precontracted tracheal rings. Tone change is given as the means ± SEM of 6 experiments

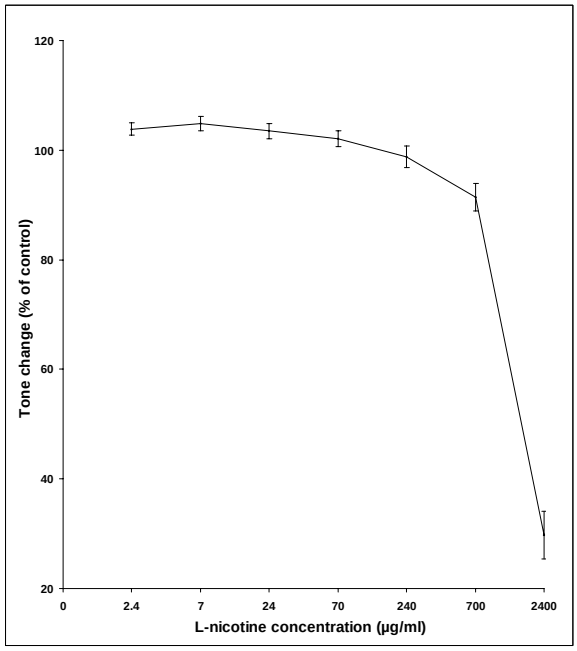

Fig. 2: Concentration-effect curve of CSE on rat phenylephrine-precontracted aorta. Tone change is given as the means ± SEM of 6 experiments

Table 1: The maximum response of rat isolated aorta and trachea preparations[a] induced by CSE

Tissue	N[b]	Maximum contraction (% of papaverine maximum)	Maximum relaxation (% of apaverine maximum)
Aorta	6	106.8 ±1.5	11.9±3.3
Trachea	6	115.9±5.8	12.2±3

Table 2: The maximum response of rat isolated aorta and trachea preparations[a] induced by L-nicotine

Tissue	N[b]	Maximum contraction (% of papaverine maximum)	Maximum relaxation (% of apaverine maximum)
Aorta	6	117.1±2.8	58.6±3.7
Trachea	6	104.9 ± 1.3	29.8 ±4.3

[a] Values are expressed as means ± SEM; [b] Number of experiments.

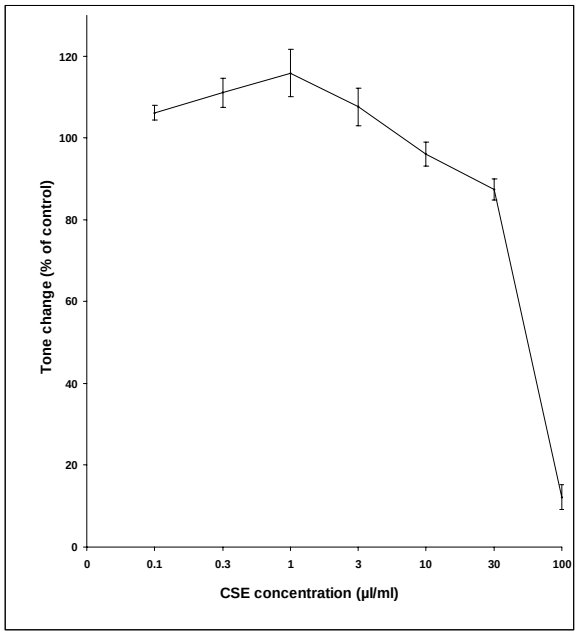

Fig. 3: Concentration-effect curve of L-nicotine on carbachol-precontracted tracheal rings. Tone change is given as the means ± SEM of 6 experiments.

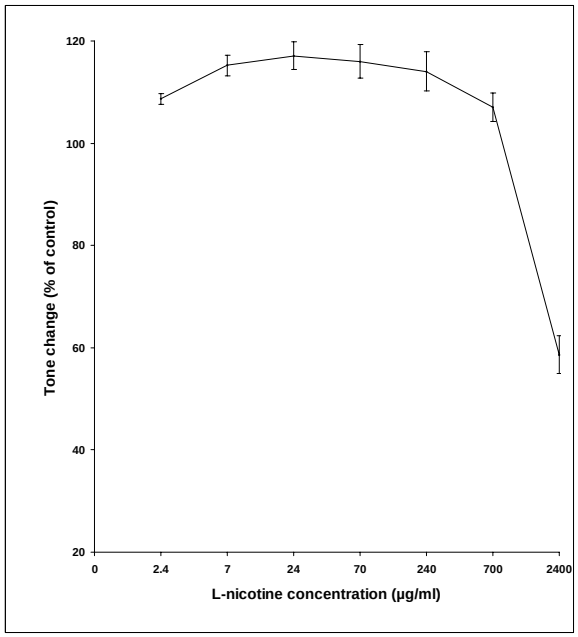

Fig. 4: Cumulative concentration-effect curve of L-nicotine on rat phenylephrine-precontracted aorta. Tone change is given as the means ± SEM of 6 experiments.

Effect of pure L-nicotine on isolated smooth muscle preparations

L-nicotine, in concentrations ranging from 2.4-2400 µg/ml induced a biphasic tension change in carbachol-precontracted tracheal rings (P<0.05) (Fig. 3 & 6). Also, L-nicotine in concentrations ranging from 2.4-2400 µg/ml caused a biphasic tension change in phenylephrine-precontracted aortic rings (P<0.05) (Fig. 4 & 6). The maximum contractile and relaxant effect of L-nicotine on isolated aorta and trachea are shown in Table 3.

CSE on the isolated perfused heart

CSE, in concentrations ranging from 0.1-30 µl/ml induced a significant concentration-dependent reduction of both the force and rate of contractions of perfused heart (P<0.05) (Fig. 7 & 9).

Effect of L-nicotine on the isolated perfused heart

L-nicotine, in concentrations ranging from 2.4-700 µl/ml caused a significant concentration-dependent decrease in the force and rate of contractions of perfused heart (P<0.05) (Fig. 8 & 10).

The effect of both CSE and L-nicotine on heart activity was not completely reversible, since washing of the chemicals failed to allow the heart to recover its pre-test base line level. This effect is clear especially at higher concentrations (10-30 µl/ml CSE in fig. 9, and 240-700 µg/ml nicotine in fig. 10). Figures 9 and 10 showed clearly that there is a cumulative effect of both CSE and L-nicotine. Their effect is not completely reversible.

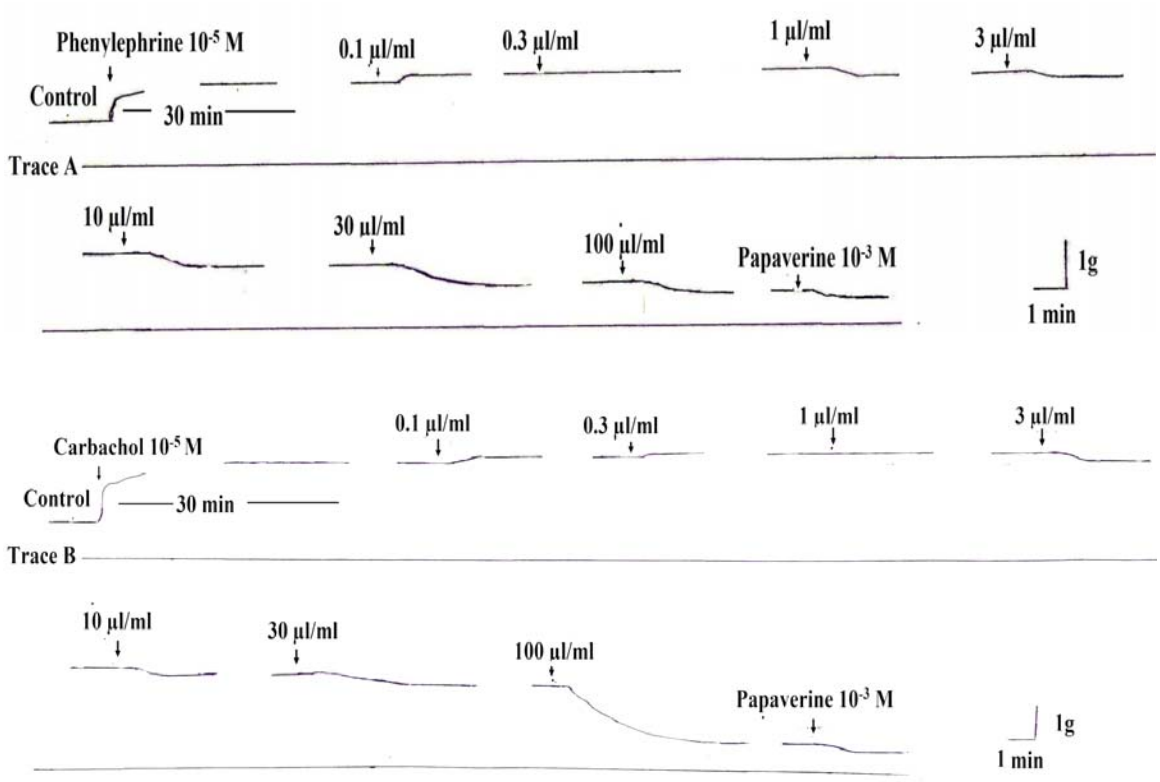

Fig. 5: Effects of various concentrations of cigarette smoke extract (CSE) on rat isolated phenylephrine-precontracted aorta (trace A), and carbachol- precontracted trachea (trace B). (Times scale is not applicable between intervals of the traces).

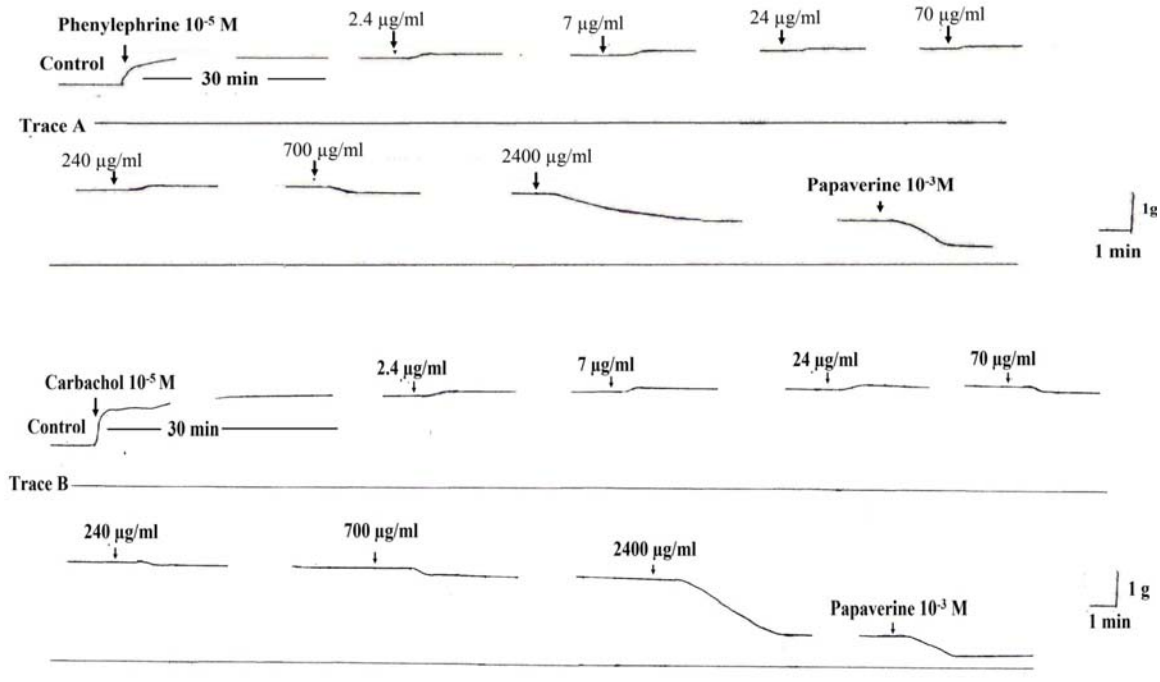

Fig. 6: Effects of various concentrations of L-nicotine on rat isolated phenylephrine-precontracted aorta (trace A), and carbachol- precontracted trachea (trace B). (Times scale is not applicable to intervals between the traces).

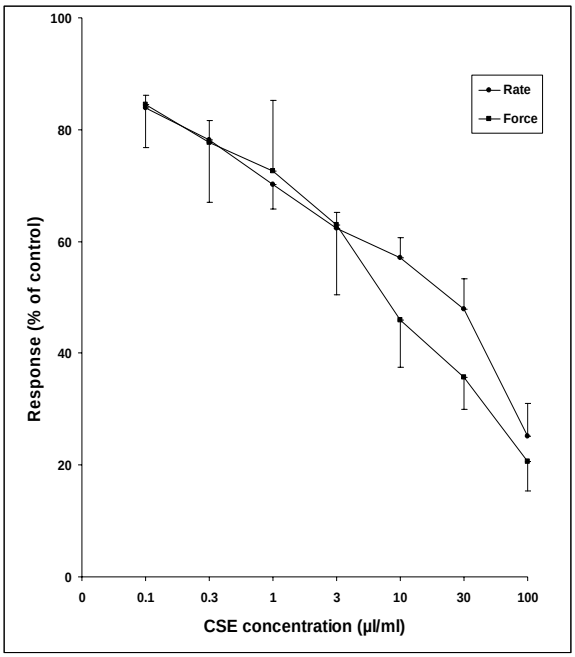

Fig. 7: Concentration-effect curve of CSE on the rate and force of rat isolated perfused heart. The force and rate of the contractions of perfused heart is given as the means ± SEM of 6 experiments

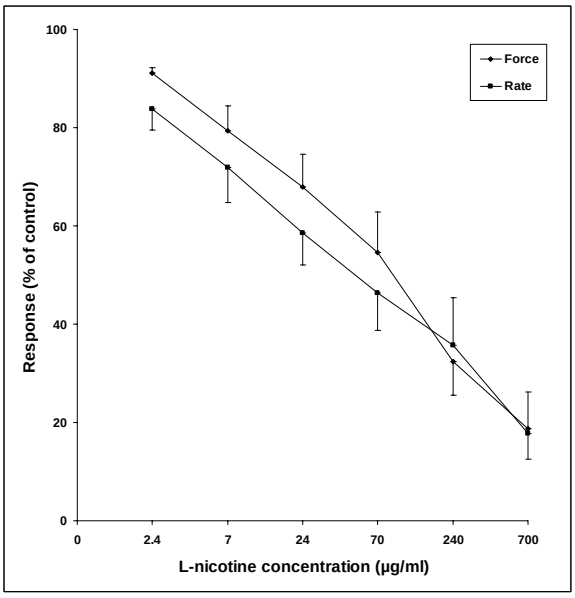

Fig. 8: Concentration-effect curve of L-nicotine on the rate and force of rat isolated perfused heart. The force and rate of the contractions of perfused heart is given as the means ± SEM of 6 experiments

Discussion

Nicotine concentration in CSE

During this work, we bubbled cigarette smoke in phosphate buffer in order to mimic what happed actually during cigarette smoking. CSE contains very low concentration of nicotine. One g tobacco produced an equivalent of 4.8 mg L-nicotine in CSE. Nicotine is a nonpolar compound, and little amount of it will remain in the phosphate buffer after bubbling of tobacco smoke through the buffer. Shafagoj et al. (2002) estimated that only about 15% of nicotine is captured by water of hubble-bubble. Hadidi and Mohammed (2004) used gas chromatography methods to determine nicotine concentration in tobacco after extraction with organic solvents. They obtained nicotine concentration range 1.8-41.3 mg/g.

Effect of CSE and L-nicotine on isolated perfused heart

In the present study, CSE induced a significant-concentration dependent reduction of the force and the rate of the perfused heart contraction. The treatment of isolated perfused rat heart with L-nicotine alone also produced a significant-concentration dependent decrease of the force and rate of the perfused heart contraction. From this result the reduction of the force and the rate of the perfused heart contraction at least partially depend on L-nicotine in CSE.

It is clear from traces B, C, D, E, and F in figure 9, and from traces C, D, and F in figure 10, that the heart rate and force of contraction were not fully recovered to the pre-test base line level after washing. This may indicate that either the accumulated doses were still working, or the effect of CSE components is not completely reversible. McGrath (1986) showed the treatment of isolated perfused rat heart by carbon monoxide did not affect the heart rate, while treating it with nicotine resulted in bradycardia. On the other hand, treating the heart with both carbon monoxide and nicotine decrease heart rate. Moreover, the separate effect of carbon monoxide and nicotine was reversible, but the effect they produced in combination was irreversible. In another study (Chen and McGrath, 1985), CO caused a contractile depression, whereas nonstimulated isolated rat hearts perfused with 95% CO-5% CO_2 (CO) KHs, were observed to depress the heart rate (McGrath, 1986). Long-term exposure of dogs to cigarette smoke or nicotine exhibited a significant deficit in heart contractility (Ahmed et al., 1976). The decrease of heart rate and force due to CSE can be explained by the presence of both nicotine and CO together, which also explains the irreversibility of the effects.

Effect of CSE on aorta

In the present study, CSE induced biphasic change in tone of isolated rat aorta. Initial contraction in response to CSE may depend partially on the degradation of basally released endothelium-derived relaxing factor (nitric oxide) by superoxide anions (Mayhan and Sharpe, 1998). The contraction may result partially from L-nicotine as in CSE; L-nicotine with the

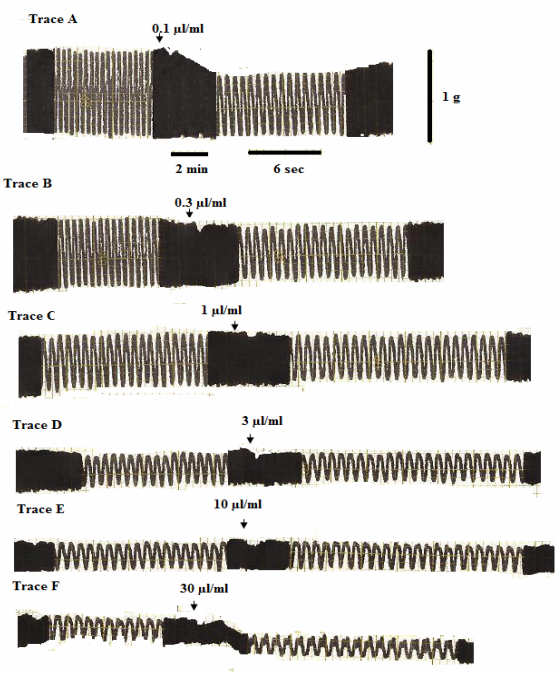

Fig. 9: Effects of increasing concentrations of cigarette smoke extract (CSE) on rat isolated perfused heart, (trace A-F). All traces are from the same preparations. The small depression immediately after the addition indicates an artifact due to rapid injection of the compound.

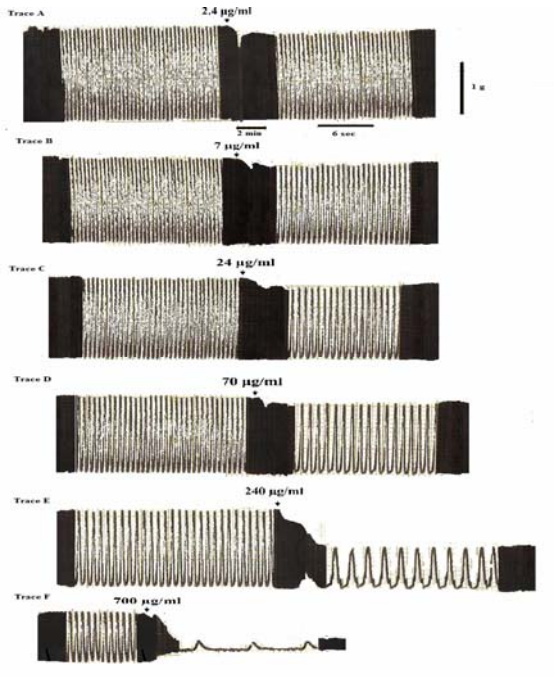

Fig. 10: Effects of increasing concentrations of L-nicotine on rat isolated perfused heart, (trace A-F). All traces are from the same preparations. The small depression immediately after the addition indicates an artifact due to rapid injection of the compound.

same concentration in CSE caused biphasic tension change on isolated precontracted aorta in the present study. The subsequent relaxation in isolated aorta also depends at least partially on L-nicotine, nitric oxide and nitrous compounds, these compounds are found in high amount in CSE (Mayhan and Sharpe, 1998).

Murohara et al. (1994) showed that CSE induced biphasic tension change in isolated pig coronary arteries during stable contraction with prostaglandin F2 alpha (PGF2 alpha). Initial contraction to CSE was dependent on the presence of endothelium, whereas subsequent relaxation was endothelium independent. The initial contraction may be, at least in part, mediated through the degradation of basally released endothelium-derived relaxing factor (nitric oxide) by superoxide anions derived from CSE. Beside the nitrous oxide production from endothelial cell, this compound was also found in high amount in cigarette smoke (Chaouachi, 2009). Cigarette smoke has been reported to induce vasodilation by nitric oxide or by nitrous compounds like N-nitrosonornicotine via stimulating vascular smooth muscle soluble guanylate cyclase (Gruetter et al., 1980).

Effect of pure L-nicotine on aorta

In the present study, L-nicotine caused biphasic tension change of isolated rat aorta. Mayhan and Sharpe (1998) suggested that nicotine impairs endothelium-dependent arterial dilation via an increase in the synthesis or release of oxygen-derived free radicals. But, the subsequent relaxation caused by L-nicotine may be mediated by NO production (Hanna, 2006).

Effect of CSE on trachea

In the present study, the CSE induced biphasic change in tone of isolated rat trachea. Schachter et al. (2003) showed that tobacco dust extract (TDE) have contractile effect on isolated guinea pig tracheal smooth muscle. The major contractile agent in this extract is not nicotine but other low-molecular-weight substances. But, in another study, the acute exposure to CSE leads to airway relaxation in mice, which was partially mediated by nicotine (Streck et al., 2010). Regarding the maximum relaxation induced by the non-selective smooth muscle relaxant papaverine, the relaxation is likely to be achieved by an intracellular accumulation of cAMP and/or cGMP by inhibiting phosphodiesterase (Kaneda et al., 2005).

Effect of pure L-nicotine on trachea

In the present study, L-nicotine with the same concentration as in CSE caused biphasic change in tone of isolated rat trachea. The initial contraction may be due to activation of nicotinic acetylcholine receptors (nAchRs) in nervous tissue by nicotine, which causes contraction through acetylcholine release from cholinergic nerves. But, the subsequent relaxation

caused by L-nicotine may be mediated by NO production (Cevit et al., 2007).

D-nicotine and L-nicotine (that is the main isomer in cigarette smoke), have different physiological effects. For instance, L-nicotine produced a biphasic response in the guinea-pig trachea, which consists of an initial contraction followed by relaxation; however, D-nicotine produced only a concentration-dependent relaxation in the tracheal preparation (Funayama et al., 1995). Our finding that L-nicotine alone produced biphasic tone change of isolated tracheal preparation agrees with the finding of Cevit et al. (2007), who found that nicotine produced a concentration-dependent relaxation on guinea-pig isolated tracheal preparations precontracted by carbachol (10^{-6} M). It was suggested that nicotine-induced relaxation is at least in part mediated by NO; since the relaxation was significantly reduced in the presence of N (w)-nitro L-arginine methyl ester (L-NAME).

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