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EFFECTS OF *JUNIPERUS COMMUNIS* AEROSOLS ON TRACHEA AND LUNG FROM RAT SUBJECTED TO PASSIVE SMOKING

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ABSTRACT

The effect of cigarette smoking and essential oil of *Juniperus communis* of tracheal contraction and dilatation was studied in 40 female Spraque-Dawley rats. The animals (rats) that were used for analysis were divided into four lots: smoking, non-smoking, exposed and not exposed to juniper oil. The smoking lot was exposed to 2 cigarettes per day, 5 days a week for 6 weeks. Then, the rats belonging to lots 2 and 4 were exposed to juniper berries oil for 3 weeks, 20 minutes per day. The control lot (lot 1) was not exposed to cigarette smoke.

In this study we also performed physiological analyses for trachea and lung of experimental rats. Histopathological studies present the effect of cigarette smoke and *Juniperus communis* oil for trachea and lung. Physiological studies were performed upon analysis of bronhodilator and bronhoconstrictor trachea effects.

Key words: juniper, rats, smoke, trachea, lung.

INTRODUCTION

The adjustment of the airway size depends on the balance between parasympathetic nervous system activation, having represented cholinergic broncho-constriction by mediation of acetylcholine and activation of the sympathetic nervous system, bronchodilator effect, through the adrenergic mediation of epinephrine represented. Both nerve pathways are distributed both in the airways tracheo-bronchial muscle fibers and the cholinergic ganglia, submucosal glands and local vascular system (Kusindarta et al., 2004, Atoji et al, 2005).

On a level with respiratory tract, there is a third kind of mediation, noncolinergic- nonadrenergic (NANC) that consists of two components: an excitatory component (NANCe), bronchoconstrictor effect induced by releasing substance P, and an inhibitory component (NANCi) with bronchodilator effect -induced by the release of nitric oxide (NO) (Mihalas et al, 2003, Ricciardolo, 2005).

Bronchial superficial nerve endings are located less than 1μm from the lumen tracheo-bronchitis. Those intraepithelial and subepithelial located fulfill the role of "irritant receptors" and are responsible for reflex bronchoconstriction mediated by substance P, in terms of exposure to all types of endo-bronchial irritants, including cigarette smoke. Excitation "irritant receptors" is the origin of an influx of transmission to the central nervous system via vagus nerve. The result is a stimulation of efferent (vagal) parasympathetic fibers that discharge acetylcholine to the tracheo-bronchial muscle fibers, causing their contraction. Substance P stimulates production of a wide range of inflammatory mediators and reactive oxygen species by resident inflammatory cells (macrophages, eosinophilia, mast cells and lymphocytes), which are thus responsible for altering the integrity of epithelium. Epithelial denudation exposure causes an increased number of "irritant receptors" and release from the injured epithelium of chemotactic factors for inflammatory cells, thus maintaining local inflammation. The release of reactive oxygen species such as superoxide anion ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (• OH) generated by the action of NAD(P)H oxidase and xanthine oxidase, increases the muscle fiber hyperreactivity on neural way, causes mucus hypersecretion, vasodilation and increased vascular permeability, unleashing and maintaining the neurogenic inflammation of the airways (Barnes, 2001).

Nitric oxide is a colorless gas with half-life in biological systems 3 to 30 msec. It is synthesized from the L-arginine amino acid by the action of nitric oxide synthase (NOS). On a level with respiratory tract are described three isoforms of (NOS), two constitutive expressed (NOSe and NOSn) and one inducibile (NOSi). NOSe isoform is expressed by endothelial cells and the produced NO has a role in the local vasodilatory response. NOSn isoform is expressed by nerve endings in the airways and the respiratory epithelium, and NO acts as a neurotransmitter produced in the NANCi path (Sekizawa et al., 1993). NOSi isoform is expressed by endothelial cells and respiratory epithelium only in the presence of proinflammatory factors and additional quantities of NO can exert dual effects on the airways (Kobzik et al, 1993). In this regard, NO is a bronchodilator factor and at the same time, a cytotoxic molecule that contributes to the destruction of epithelium and to inducing reflex

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bronchoconstriction. NO cytotoxicity occurs in the presence of some oxidants factors, such as those contained in cigarette smoke, which generates reactive species of nitric oxide: nitric oxide ($NO^{\bullet}$), nitrosonium cation (NO^{+}), nitroxyl anion (NO^{-}) and peroxynitrite ($ONOO^{-}$) (Kuo and Schroeder, 1995).

The interest in the biological effects of essential oils has grown considerably in recent years. Among these, there is also juniper oil, a natural product widely used in food and pharmaceutical industry, and in perfumery and cosmetics. Experimental models in vitro, place juniper oil on the list of essential oils with anti-inflammatory and antioxidant properties. In this regard, a component of Juniper oil inhibits the production of arachidonic acid metabolites, proinflammatory cytokine production and proinflammatory gene expression and / or act as scavenger of reactive oxygen species and nitric oxide (Maria GM, 2010).

Our study sought to evaluate *anti-inflammatory and antioxidant properties* of juniper oil in the tracheo-bronchial airways of rats and covered the following aspects: use of a method of direct exposure to respiratory juniper oil administered by nebulization, a standardized experimental model chronic obstructive pulmonary disease (COPD) induced by chronic exposure of rats to cigarette smoke, a standardized experimental model in vitro study in isolated organ bath, tracheal ring reactivity and morphological study classical model (standard staining with hematoxylin - eosin) fragments of the trachea and lungs.

MATERIALS AND METHODS

Plant material, solutions and chemicals

The plant material was collected from wild growing *Juniperus communis* shrubby trees from Albac (Romania) region in October 2009. One kind of sample was selected: black mature berries that were dried at room temperature.

For extract drying, anhydrous Na_2SO_4 (Fluka) is used.

Isolation of essential oil

The berries were milled prior to hydrodistillation. The berries-to-water ratio was 1:10 (w/v). Samples of 50g crushed berries with 500ml distilled water were hidrodistillated for 3h (Butkienė et al., 2009, Cavaleiro et al., 2003, Sezik et al., 2005, Shahmir et al., 2003, Tunalier et al., 2002). The hydrodistillation apparatus was consisting of two flasks (one for distillation and other for essential oil collection), a condenser and than was using an essential oil separator. Lighter than water, the slightly yellow oil was dried over anhydrous Na_2SO_4 and stored in sealed dark-glass containers in a freezer (4^0C) until analysis.

Preparation of the lot

The study was conducted on a 40 Spraque-Dawley rats (female) lot, weighing between 200 and 300 grams, coming from UMF "Victor Babes" Biobase Timisoara, who were divided into four lots:

- Lot 1 (n = 10) - rats that were not exposed to tobacco smoke and / or juniper oil
- Lot 2 (n = 10) - rats that were exposed to juniper oil
- Lot 3 (n = 10) - rats exposed to cigarette smoke
- Lot 4 (n = 10) - rats initially exposed to cigarette smoke then to the juniper oil

Throughout the experiment, rats received standard conditions of growth and development, respectively 22°C temperature, 55% humidity, 12-hour circadian light / dark, and had *ad libitum* access to water and standard food (Cantacuzino Institute, Bucharest).

Experiments were conducted according to current regulations on animal protection.

Chronic exposure to cigarette smoke

Rats were placed in a sealed glass chamber with volume of about 20 liters, with: a "device" for the automatic generation of smoke, consisting of a vacuum pump driven by an airflow compressed with a 3 L/min pressure flow, 0.12 MPa, and an outlet for excess air and cigarette smoke, with the purpose to avoid excessive pressure inside the exposure.

Air flow generated under sub-atmospheric pressure provided 'smoking' the cigarette attached to the exterior enclosure system of exposure and at the same time, mainstream training of cigarette smoke exposure results in the enclosure.

Rats belonging to lots 3 and 4 were exposed to cigarette smoke for 6 weeks, 5 days a week. For the exposure were used for two filtered cigarettes each per day, containing 0.8 mg tar / cigarette and 0.6 mg nicotine / cigarette. Each cigarette was 'smoked' within 10 min. After each exposure, rats were allowed to breathe the atmosphere of the chamber for 30 min.

Conventionally, cigarette smoke contains two phases: tar and gas phase. Tar phase is defined as the material retained when the main stream is passed through a Cambridge glass fiber filter. This phase comprises 99.9% of the material particle size of > 0.1 μm and > 10^{17} free radicals / g. Gaseous phase is passing particle filter material comprises > 10^{15} Cambridge and free radicals / puff (Munteanu and Didilescu, 2007).

Exposure to oil juniper

Rats belonging to lots 2 and 4 were exposed to juniper oil for 3 weeks, 5 days a week. At each exposure lasting 20 min in sealed glass chamber nebulized volume was 1.84 ml / day volatile oil obtained by *Juniperus communis* hydrodistillated berries. After each exposure

rats were allowed to breathe the atmosphere of the chamber for 20 min. To manage juniper oil, continuous flow of 5-6 L / min 'Pari – Boy" type nebulizer was used (Welch Allyn, Germany)

Getting tracheal smooth muscle ring

The animals were killed by intraperitoneal administration of thiopental sodium at high doses of 50 mg / kg, followed by cervical dislocation. After slaughter, the thoracic cavity was opened through an incision in the chest and the trachea was isolated, from which tracheal smooth muscle rings were sectioned length 2 - 2.5 mm.

Study of tracheal smooth muscle ring reactivity

Tracheal smooth muscle rings (MNT), previously fixed on a fastening system have been introduced in a organ baths with a volume of 10 mL, an installation BIOPAC MP 100 (BIOPAC System Inc., USA) fitted with a transducer isometric force and a manual micrometer control of the extent of preparation. Isometric tension generated during the experiment MNT rings was measured continuously by a Fort 10g isometric force transducer (World Precision Instruments Inc.). Data were amplified, acquired and stored in computer memory using a hardware interface MP 100 of a software program dedicated BIOPAC AcqKnowledge, version 3.7.2 (BIOPAC System Inc. USA).

Reagents

To obtain the working solutions, pure reagents were used, from which appropriate dilutions were prepared. The following reagents were used:

- *Acetylcholine chloride* (ACh) - selective agonist of muscarinic receptors M1 - M3, Sigma Product Code A6625
- *Epinephine jump bitartrate* (EPI) - a non-selective receptor agonist α1 and β2 - adrenergic, Sigma Product Code E4375
- *Sodium nitroprusside dihydrate* (NPS) - donor of nitric oxide (NO), Product Code 71778 (Fluka)
- *Pirenzepine dihydrochloride* (PIR) - selective blocker of muscarinic M1 receptors, Sigma Product Code P7412

Pure substances were dissolved in distilled water, except pirenzepine which was dissolved in a solution of 50% ethanol. The formula for calculating the volume of working solution used to obtain the desired concentration in the organ bath was as follows:

$$V = \frac{C_B \times (0 - v)}{C_S - C_B}$$

where:

V = volume of working solution used,
C_S = the working solution concentration,
C_B = desired concentration in the organ bath,
10 = organ bath volume (mL),
v = volume of liquid displaced by the organ bath device vascular clamp preparation.

Viable Krebs - Henseleit solution

To maintain viability in the organ bath preparation, a viable Krebs - Henseleit solution was used, with 37°C temperature and continuously aerated with a mixture of 95% O_2 and 5% CO_2. Krebs-Henseleit solution used had the following composition: NaCl 118 mM, KCl 4.7 mM, KH_2PO_4 1.2 mM, $MgSO_4$ 1.2 mM, $CaCl_2$ 2.5 mM, $NaHCO_3$ 25 mM, glucose 11.1 mM, Na_2EDTA 0.026 mM. pH was checked throughout the experiment at intervals of 30 minutes (pH = 7.4).

MNT rings were pre tensioned at 1.5 g force and were balanced for 60 minutes. Throughout the balance, preparations were washed with sodium Krebs - Henseleit every 15 min.

Stages of experiment

I. Balancing the preparation was verified by obtaining two similar contractile responses to acetylcholine 10^{-5} M (the difference between the forces generated by the two successive contractions < 10-15%).
II. MNT contractile response of rings was evaluated by determining the dose-response curve to acetylcholine, cumulative doses ranging from 10^{-7} M and 10^{-4} M (Fig. 1) and contraction force generated was expressed in absolute (cN).
III. MNT relaxant response of rings was evaluated by determining the dose-response curves to epinephrine (Fig. 2), sodium nitroprusside (Fig. 3) and in pirenzepine (Fig. 4), using the cumulative dose range for each 10^{-7} M and 10^{-5} M. The curves were obtained with acetylcholine pre-contraction background, sub-maximal dose of 10^{-5}M, and the result was expressed as % relaxation of pre-contraction induced by it.

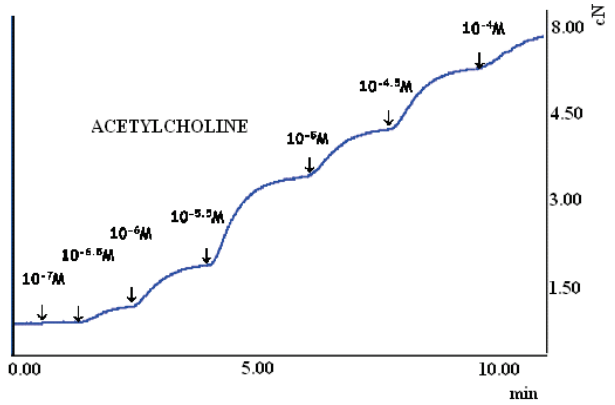

Fig. 1 Work schedule: dose - effect curve to acetylcholine (10^{-7}M - 10^{-4}M

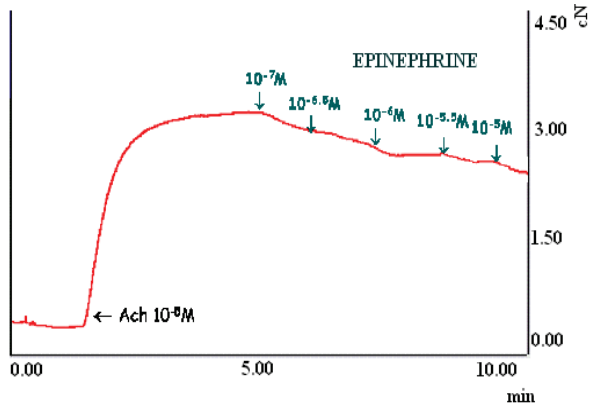

Fig. 2 Work schedule: dose - effect curve to epinephrine (10^{-7}M - 10^{-5}M)

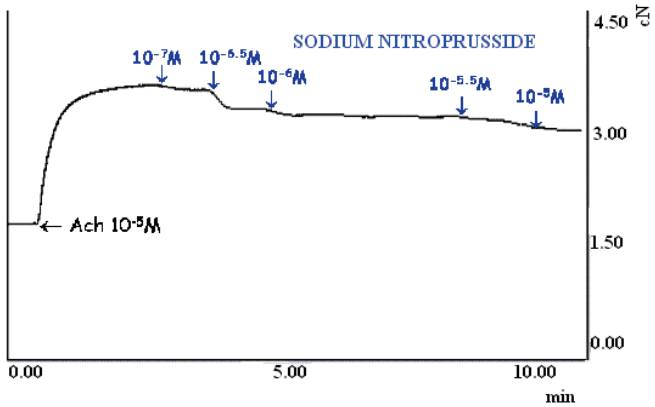

Fig. 3 Work schedule: dose - effect curve of sodium nitroprusside (10^{-7}M - 10^{-5}M)

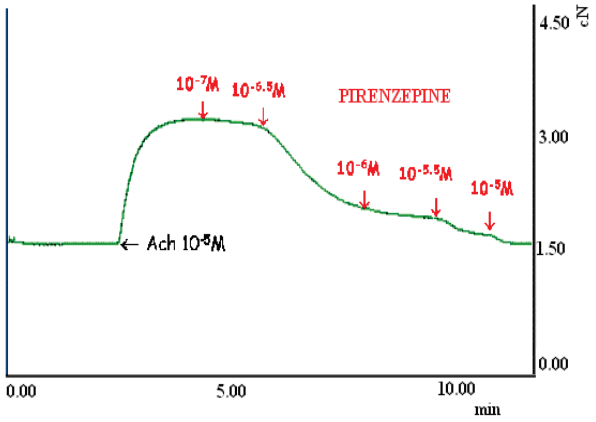

Fig. 4 Work schedule: dose - effect curve for pirenzepine (10^{-7}M- 10^{5}M

Histopathological exam

Fragments of trachea and lung, harvested immediately after animal slaughter, were processed by fixing in 10% formaldehyde, embedding and sectioning at 4-5 mm (serial sections were performed on average 4-5 sections / case). Morphological study was performed on sections stained with hematoxylin-eosin (standard technique) applied to all studied cases.

Statistical analysis

Acquired data were statistically analyzed using dedicated software program GraphPad Prism 5 (GraphPad Software, USA). Central tendencies of the variables obtained from *n* different rings were expressed as mean (M) and the dispersion as the standard error (SE). Normal distribution, Gaussian type, of the variables in the series of analyzed values, was confirmed using the Kolmogorov - Smirnov test. This test for assessing the normality of distribution of a range of values, was chosen, due to the relatively small number of data obtained from a sample.

In order to assess the overall responsiveness MNT ring, it was chosen to calculate several variables of Hill equation for a sigmoidal-type relationship, and for comparing curves doses - reply to this equation was estimated using F-test used.

The parameters used to assess overall reactivity MNT ring have the following meanings:

- **Bottom** - the estimated minimum response
- **Top** - estimated maximum response
- **EC_{50} (-log [M])** - the concentration required to achieve 50% of maximum contractile response
- **IC_{50} (-log [M])** - the concentration required to achieve 50% of the maximum relaxant response
- **Hill Slope** - the slope of the dose-response curve

RESULTS AND DISCUSSION

Results of global assessment parameters MNT contractile response of rat to cumulative doses of acetylcholine are shown in Table I.

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Table I. Global assessment of parameters of MNT contractile response of rat to cumulative doses of acetylcholine, estimated by Hill equation with four variables

	Lot 1	Lot 2	Lot 3	Lot 4	P
Bottom	- 0,16 ± 0,30	- 0,23 ± 0,26	- 0,12 ± 0,27	- 0,97 ± 0,72	< **0,001**
Top	2,88 ± 0,28	3,59 ± 0,25	3,00 ± 0,23	5,43 ± 0,44	
LogEC$_{50}$	- 5,5 ± 0,14	- 5,51 ± 0,10	- 5,56 ± 0,12	- 5,76 ± 0,14	
Hill Slope	0,74 ± 0,23	0,73 ± 0,16	0,76 ± 0,20	0,57 ± 0,14	

Note: Data are presented as mean ± standard error

From the analysis of dose – effect curves, it results that the contractile response to acetylcholine MNT rings was higher in rats lots chronic exposed to juniper oil, the difference being statistically significant in lot 2 compared with lot 1 ($p < 0.001$), and the lot 4 compared with lot 3 ($p < 0.001$) as well. Although the contractile response to acetylcholine was higher in lot 3, chronically exposed to cigarette smoke, compared with lot 1, the difference was not statistically significant ($p = 0.51$). The largest contractile response to acetylcholine was obtained in lot 4, the difference being statistically significant compared with lot 1 ($P < 0.001$) and lot 2 ($p < 0.001$) (Fig. 5).

Results on the parameters of the overall assessment of contractile response in rat MNT cumulative doses of epinephrine are presented in Table II.

Table II. Global assessment parameters of the relaxing response (% of pre-contract with 10^{-5}M acetylcholine) of the rat MNT cumulative doses of epinephrine, estimated by Hill equation with four variables

	Lot 1	Lot 2	Lot 3	Lot 4	P
Bottom	- 0,36 ± 2,49	-2,83 ± 5,5	1,64 ± 1,11	1,15 ± 2,27	< **0,001**
Top	30,78 ± 1,29	25,85 ± 2,18	13,64 ± 0,82	16 ± 1,55	
LogIC$_{50}$	- 6,26 ± 0,07	- 6,32 ± 0,16	- 6,12 ± 0,09	-6,15 ± 0,14	
Hill Slope	1,44 ± 0,31	1,02 ± 0,39	2,078 ± 0,83	1,9 ± 1,13	

Note: Data are presented as mean ± standard error

From the analysis of dose - effect curves, it results that relaxing response to epinephrine decreased significantly in lot 2 compared with lot 1 ($p < 0.001$) and in lot 3 compared with lot 1 ($p < 0.001$).

Although it has increased in lot 4 compared with lot 3 ($p < 0.001$), the relaxant response to epinephrine remained significantly lower compared with lot 1 ($p < 0.001$) (Fig. 6).

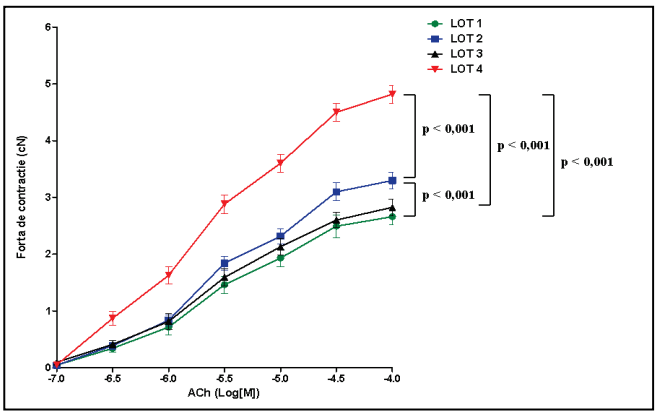

Fig. 5 Graphical representation of dose - effect curves to acetylcholine (10^{-7}M - 10^{-4}M) for all lots studied

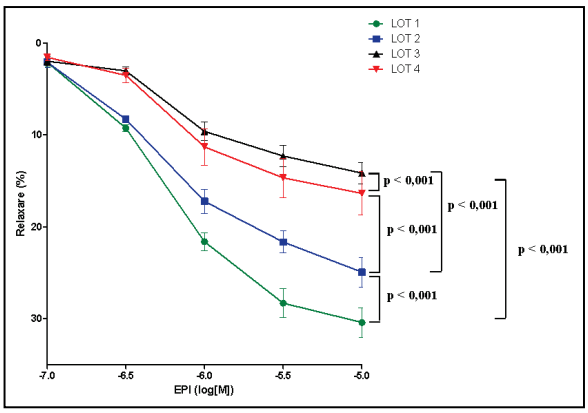

Fig. 6 Graphical representation of dose - effect curves for epinephrine (10^{-7}M - 10^{-4}M) for all lots studied

Results regarding the parameters of the overall assessment of contractile response of rat MNT cumulative doses of sodium nitroprusside are shown in Table III.

Table III. Global assessment parameters of the relaxing response (% of pre-contract with 10^{-5}M acetylcholine) of the rat MNT cumulative doses of sodium nitroprusside, estimated by Hill equation with four variables

	Lot 1	Lot 2	Lot 3	Lot 4	P
Bottom	0,14 ± 1,40	-0,48 ± 3,97	1,06 ± 0,55	-2,78 ± 4,48	< **0,001**
Top	26,09 ± 1,28	29,72 ± 1,52	21,63 ± 0,72	27,9 ± 1,89	
LogIC$_{50}$	- 6,03 ± 0,05	- 6,42 ± 0,09	- 5,90 ± 0,03	-6,31 ± 0,12	
Hill Slope	1,35 ± 0,26	1,73 ± 0,60	1,84 ± 0,26	1,19 ± 0,39	

Note: Data are presented as mean ± standard error

Upon the analysis of dose effect curves, it results that relaxing response to sodium nitroprusside increased in chronically exposed to lots of juniper oil, the difference being statistically significant for lot 2 ($p < 0.001$) compared with lot 1, as well in lot 4 compared with lot 3 ($p < 0.001$). Rings MNT relaxant response to sodium nitroprusside was lower with lots of rats exposed to chronic cigarette smoke, the difference being statistically significant in lot 3 compared with lot 1 ($p < 0.001$). The largest of the relaxing response to sodium nitroprusside was obtained in lot 2, the difference was significantly higher compared with lot 3 ($p = 0.003$) and lot 4 ($p = 0.003$) (Fig. 7).

Results on the parameters of the overall assessment of contractile response of rat MNT pirenzepine cumulative doses are presented in Table IV.

Table IV. Global assessment parameters relaxing response (% of pre-contract with 10^{-5}M acetylcholine) of the rat MNT pirenzepine cumulative doses estimated using Hill equation with four variables

	Lot 1	Lot 2	Lot 3	Lot 4	P
Bottom	0,77 ± 2,19	-0,40 ± 2,31	0,34 ± 2,81	- 5,5 ± 5,39	< **0,001**
Top	95,68 ± 1,52	95,36 ± 1,62	96,20 ± 2,10	103,9 ± 5,40	
LogIC$_{50}$	- 6,17 ± 0,02	- 6,15 ± 0,02	- 6,10 ±0,02	-5,99 ± 0,05	
Hill Slope	3,00 ± 0,32	2,24 ± 0,22	2,34 ± 0,34	1,22 ± 0,21	

Note: Data are presented as mean ± standard error

By analyzing the dose - effect response curves, it resulted that relaxing response for pirenzepine fell down for chronically exposed to juniper oil lots, the difference being statistically significant for lot 2 ($p = 0.04$) compared with lot 1, and in lot 4 compared with lot 3 ($p = 0.002$). Relaxant response of rings to pirenzepine MNT was lower in the case of rats lots exposed to chronic cigarette smoke, the difference being statistically significant in lot 3 compared with lot 1 ($p = 0.005$). The smallest relaxing response was obtained for pirenzepine in lot 4, the difference being statistically significant compared with lot 1 ($p < 0.001$) and lot 2 ($p < 0.001$) (Fig. 8).

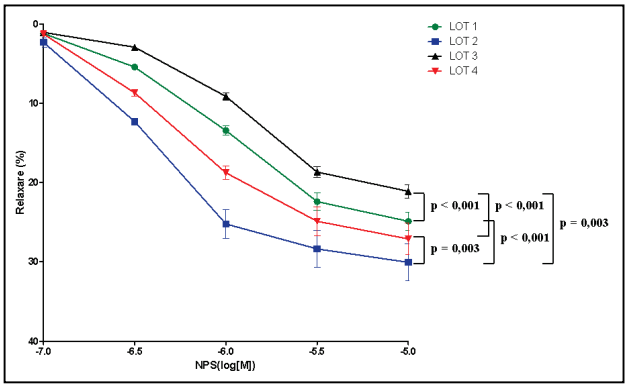

Fig. 7 Graphical representation of curves in dose - effect of sodium nitroprusside (10^{-7}M - 10^{-4}M) for all lots studied

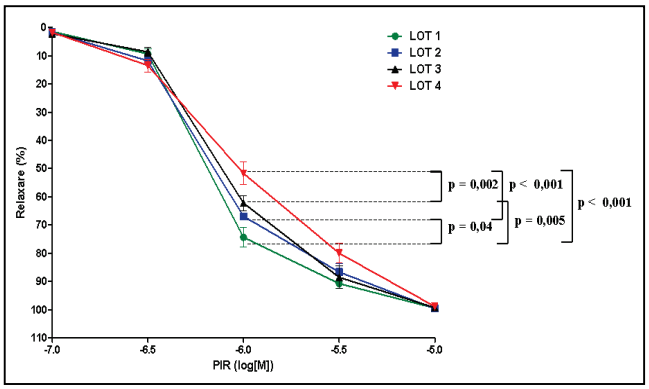

Fig. 8 Graphical representation of dose - effect curves for pirenzepine (10^{-7}M - 10^{-4}M) for all lots studied

Histopathological exam

Histopathological changes induced by cigarette smoke and / or exposure to oil of juniper on the trachea and lung parenchyma, by the sections stained with hematoxylin-eosin (standard technique) applied to all studied biologicals, were evaluated.

Exposure to cigarette smoke (Lot 3)

The main changes induced by chronic exposure to cigarette smoke, in the case of trachea biologicals, were: presence of extensive areas of exposure of the respiratory epithelium, alternating with an uneven thickening of the epithelium, calciform cell hyperplasia in respiratory epithelium, edema of lamina propria, with venulo-

capillary congestion, polymorphous inflammatory infiltrate, respectively, subepitelial located and perivascular, where the presence of macrophages was noted, and the presence of suppurative micro pest holes extended on full thickness of trachea and peritracheal (Fig. 9, Fig.10, Fig.11).

The main changes induced by chronic exposure to cigarette smoke for lung biologicals were: the presence of pulmonary emphysema pest holes, alternating with slightly collapsed lung alveoli, and emphysema predominantly panacinar, generalized or more subpleural prominent, with alveolar ducts and relaxed alveoli, and thin alveolar septa broken in patches (Fig.12, Fig. 13).

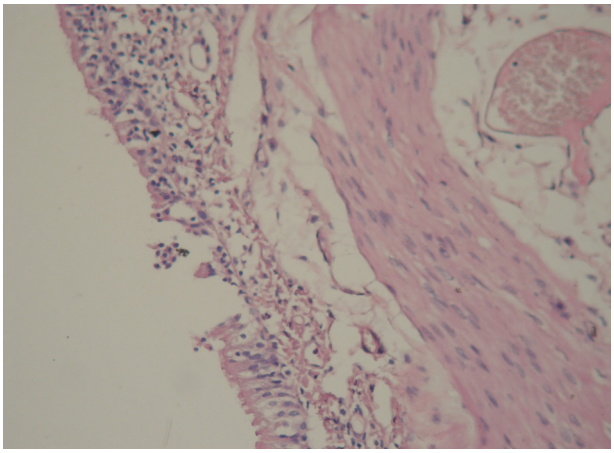

Fig. 9 Section through the trachea. Rat lots exposed to cigarette smoke. Wide area of pest hole ulceration of the respiratory epithelium, abundant and perivascular inflammatory infiltrate subepitelial (col. HE x 200)

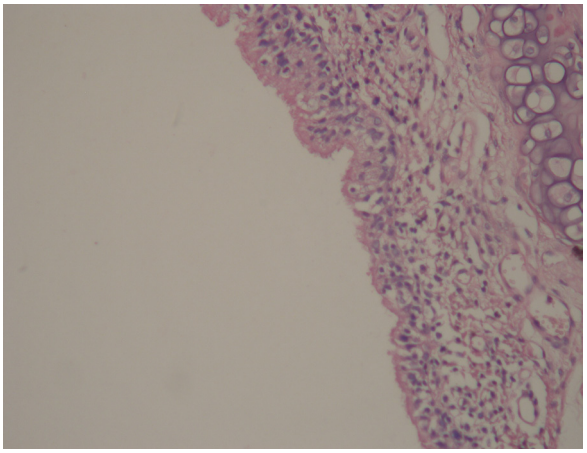

Fig. 10 Section through the trachea. Rat lots exposed to cigarette smoke. Tracheal mucosa with irregular thickening of the epithelium, intraepithelial lymphocytes in the migration, and perivascular inflammatory infiltrate subepitelial multiforme, venulo-capillary congestion in the lamina propria (col. HE x 400)

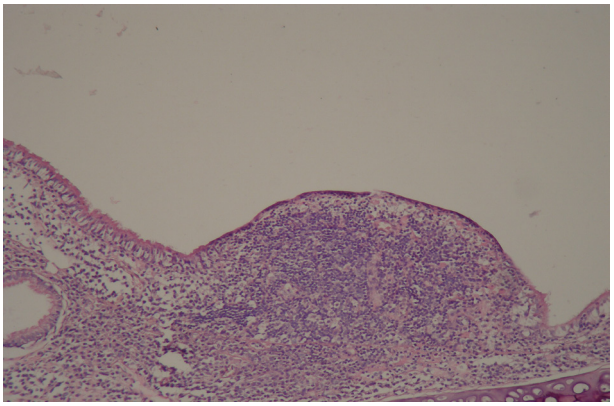

Fig. 11 Section through the trachea. Rat lots exposed to cigarette smoke. Extensive ulceration of the mucosal epithelial hyperplasia of lymphoid tissue in the law, which notes macrophages calciform cell hyperplasia in respiratory epithelium, submucosa with polymorphous inflammatory infiltrate, extensive full thickness micro pest holes suppurative trachea and peritraheal (col. HE x 200)

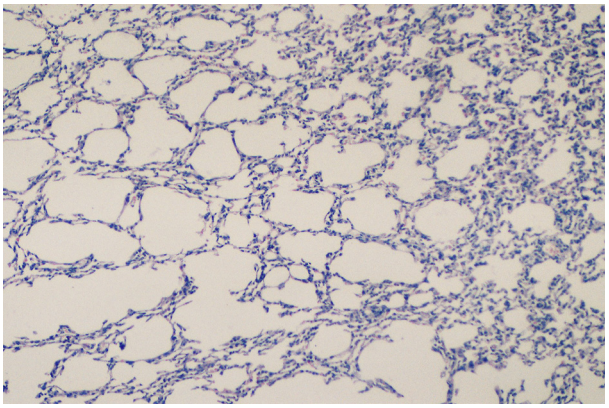

Fig. 12 Section through the lungs. Rat lots exposed to cigarette smoke. Pest holes of pulmonary emphysema alternating with slightly collapsed alveoli (HE x 200)

Juniper oil exposure (Lot 2)

The main changes induced by chronic exposure to oil of juniper, in the case of the trachea biologicals were: discrete areas of mucosal epithelium ulceration, discrete aspects of chronic tracheitis, non-specific, with moderate fibrosis of the lamina propria and submucous, polymorphous inflammatory infiltrate in the tracheal mucosa (Fig.14, Fig.15, Fig.16).

Exposure to tobacco smoke and juniper oil (Lot 4)

No significant differences were observed between histopathological changes in trachea and lung biologicals obtained in lot 4 and lot 3.

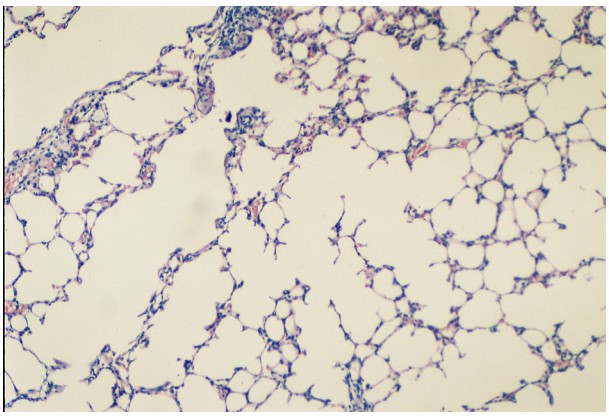

Fig. 13 Section through the lungs. Rat lots exposed to cigarette smoke. Panacinar emphysema. Alveolar ducts and relaxed alveoli, thin, broken alveolar septa (col.HE x 200)

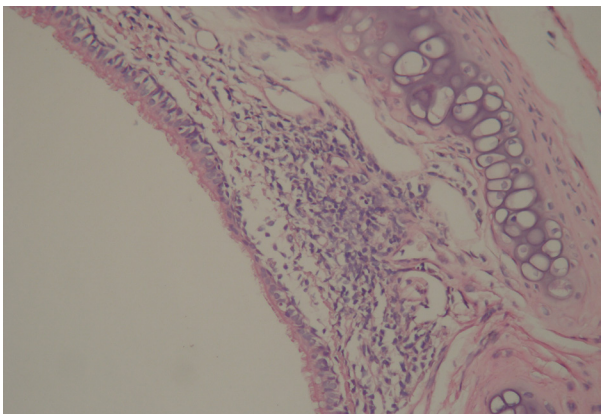

Fig. 14 Section through the trachea. Rats of the lot exposed to juniper oil. Discreet mucosal epithelium ulceration area next to a diffuse lymphoid proliferation in the lamina propria (col. HE x 400)

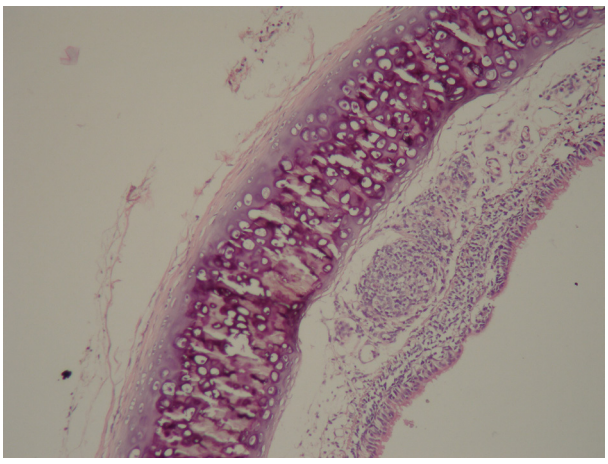

Fig. 15 Section through the trachea. Rats of the lot exposed to juniper oil. Discrete aspects of chronic tracheitis, nonspecific, with accumulation of lymphoid tissue and moderate fibrosis of the lamina propria and submucosal (col. HE x 100).

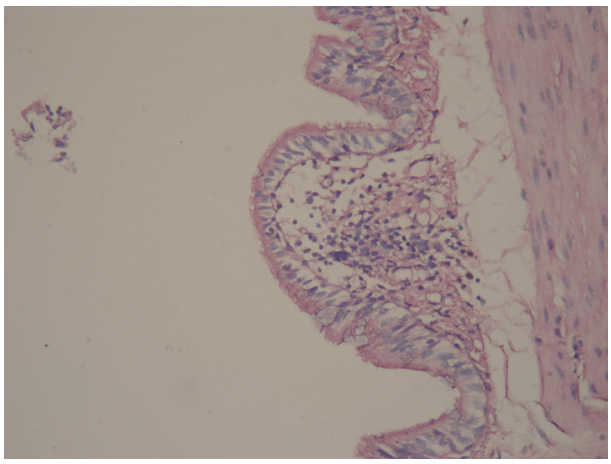

Fig. 16 Section through the trachea. Rats of the lot exposed to oil of juniper. Pseudopolipoid transformation of tracheal mucosa with connective axis, edematiate with polymorphous inflammatory infiltrate (HE x col. 200).

CONCLUSION

Juniper oil components, administered by nebulization, have had an "irritating" effect on the airway mucosa in rats and resulted in "neurogenic inflammation" of the tracheo-bronchial tract, characterized by functional smooth muscle ring tracheal hyperreactivity to acetylcholine and sustained morphological by tracheal epithelial lesions and the presence of inflammatory infiltrate.

Chronic exposure to juniper oil, administered by nebulization resulted in altered physiological mechanisms of neurogenic regulation of tracheo-bronchial tone, with the emergence of "disbalance" component of sympathetic, parasympathetic component bronchodilators, bronchoconstriction, respectively "disbalance" of the sympathetic component β2 - adrenergic, bronchodilators, and sympathetic component α 1 - adrenergic, bronchoconstriction.

On the background of preexisting inflammatory airway lesions induced by chronic exposure to cigarette smoke components of oil of juniper, administered by nebulization, have boosted the response bronchoconstrictor ring tracheal smooth muscle, assessed in isolated organ bath, and have not influenced the inflammatory infiltrate, as evidenced by morphological study, from its level.

Juniper oil components, administered by nebulization, increased the "bioactivity" of nitric oxide

in the pipes of rats, both under normal and of preexisting lesions conditions induced by chronic exposure to cigarette smoke.

Juniper oil components exercise a positive, antioxidant effect, on one hand, which facilitates the bronchodilator response mediated by nitric oxide in respiratory tract of rats, and, on the other hand, carries negative effects, irritative and pro-inflammatory, determined most likely by the route of administration by nebulization.

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