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Pistacia lentiscus L. and airway smooth muscle

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ABSTRACT

The effect of *Pistacia lentiscus* L. leaf extracts (aqueous, 30% ethanol and 70% ethanol) was studied on the rat isolated trachea. *Pistacia lentiscus* L. extracts induced a relaxation on basal tone and precontracted trachea. On the rat isolated trachea precontracted by acetylcholine 10^{-3}M, *Pistacia lenticus* L. leaf extracts (aqueous, 30% ethanol and 70% ethanol) induced a relaxation. EC_{50} values for the 70% ethanol, 30% ethanol and aqueous extracts were respectively: $1.00.10^{-3} \pm 0.11$, (n = 15), $1.71.10^{-3} \pm 0.09$ (n = 8) and $1.75.10^{-3} \pm 0.12$ g/ml (n = 12). The relaxation to *Pistacia lentiscus* L. 70% ethanol extract was 182.14 ± 17.52% (n = 15) of the response to theophylline 3.10^{-3}M. Atropine, propranolol, phentolamine (10^{-7}M) and indomethacin (10^{-6}M) did not modify *Pistacia lentiscus* L. induced relaxation. Forskolin (10^{-6}M) and methylene blue (3.10^{-5}M) respectively potentiated and antagonized *Pistacia lentiscus* L. relaxation. The studied extract (10^{-6} to 10^{-4} g/ml) did not affect theophylline, IBMX (3-isobutyl-1-methylxantine, nifedipine, dipyridamole, rolipram and siguazodam concentration-response curves but caused in a dose dependent manner left shift to those of captopril, sodium nitroprussiate, 8 phenyltheophylline and enprofylline. Moreover, the extract induced total reversal of the zaprinast-induced relaxation. On precontracted rat trachea, adenosine induced contractile response and predominant relaxation. *Pistacia lentiscus* L. blocked adenosine contractile response and potentiated the purine nucleosie relaxation. It is suggested that the recorded relaxation induced by the studied drug involves several mechanisms of action including an inhibition of angiotensin I converting enzyme (ACE), an interaction with phosphodiesterase (PDE) V and an interaction at adenosine receptors level.

KEYWORDS

Pistacia lentiscus L.,
Rat isolated trachea;
Angiotensin I converting enzyme;
Phosphodiesterase inhibitors;
Adenosine.

INTRODUCTION

Pisticia lenticus L. (Anacardiacea) is widely distributed in the Mediterranean area[3]. Mastic obtained from *Pistacia lenticus* L. was shown to be effective in the treatment of benign gastric[11] and duodenal ulcers[1]. Mastic was also shown to be cytoprotective and mild antisecretory on induced gastric and duodenal ulcers in rats[2]. It has also been demonstrated that the mastic possesses antibacterial activity against Helicobacter py-

lori[12,18]. However, Loughlin et al.,[16] demonstrated that monotherapy with mastic does not eradicate Heliobacter pylory infection from mice. Recently, it has been shown that *Pistacia lentiscus* was effective in suppressing iron-induced lipid peroxidation[15].

Essential oils of leaves were found to be rich in monoterpene hydrocarbons[9]. Recently, Vaya and Mahmood, 2006[32] demonstrated the presence of the isoflavone genistein in the Pistacia leaf. The aerial part of *Pistacia lenticus* L. is used in folk medicine against hypertension[23]. A polymeric procyanidin fraction with hypotensive activity has been isolated from *Pistacia lenticus* L.[23]. It has been suggested a possible involvement of the angiotensin system [inhibition of angiotensin I converting enzyme (ACE)] as the mechanism of action of the recorded hypotensive effect of *Pisticia lentiscus* L.[4,24].

It is reported that *Pistacia lentiscus* L. leaves and young branches are used in jaundice and respiratory problems[15]. Since the plant can affect broncho-pulmonary tract, the present work was undertaken to test the effect of the drug on airway smooth muscle.

MATERIALS AND METHODS

Plant material

Leaves of *Pistacia lenticus* L. were collected in Tunisia, air dried, powdered and extracted with distilled water, 30% ethanol and 70% ethanol. The aqueous and ethanol extracts were centrifuged, filtered and lyophilized.

Rat isolated trachea

Male rats weighting from 300 to 400g were obtained in a homogeneous breeding center (Central Animal House, Tunisia Pharmaceutical Industries Society). They were killed by a blow and exsanguinated. The tracheas were rapidly removed and cut into segments of 3-4 rings. Individual tissues were suspended under an applied load of 1.5 g in 10 ml organ baths containing Krebs-Henseleit solution at 37^0C and gassed continuously with 95% O_2 + 5% CO_2. The composition of the Krebs-Henseleit solution was (mM): NaCl 114, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.00, glucose 11.7. Tension was measured isometrically with Ugo Basil strain gauges displayed on Geminy Ugo Basile recorders (Italy).

Protocols

After equilibration for 1.25h with wash every 15 min, each experiment was initiated to maximal tension with acetylcholine (ACh 3.10^{-3}M), and then maximal relaxation was obtained with theophylline (THEO 3.10^{-3}M). The tissues were washed several times to re-establish base line tension. Concentration-response curves were obtained in preparations precontracted with acetylcholine 10^{-3}M or 10^{-5}M. After a stable level of precon traction was achieved, the agents were added to the bath in cumulative concentrations. Afterwards, theophylline 3.10^{-3}M was added to the bath to obtain maximal relaxation. Pretreatments were performed by addition of the agents30 mn. before acetylcholine was added.

Drugs

The drugs used were: acetylcholine HCl, adenosine, propranolol, atropine sulphate, captopril, forskolin, 3-isobutyl-1-methylxanthine (IBMX), 8 phenyltheophyl line, enprofylline (3-propylxanthine) (Sigma, St Louis, USA), nifedipine (Dolder), theophylline sodium anisate, methylene blue, indomethacin, NaF, sodium nitroprusside (Siphat, Tunisia), phentolamine methane sulphonate (Ciba), isoprenaline sulfate (Acros), rolipram, siguazodam, zaprinast (Laboratoire de Pharmacologie, Faculté de Médecine Paris Ouest) . With the exception of theophylline and phentolamine which were used as proprietary injectable solutions (Siphat and Ciba), all substances were in powder form. Propranolol, atropine, captopril, IBMX, methylene blue, NaF, isoprenaline and sodium nitroprusside were dissolved in Krebs solution. Adenosine, dipyridamole, 8 phenyltheophylline, enprofylline, nifedipine, indomethacin, rolipram, forskolin, siguazodam and zaprinast were dissolved in dimethylsulphoxide (DMSO). Forskolin, siguazodam and zaprinast were dissolved in dimethylsulphoxide (DMSO). The concentration of alcohol and dimethylsulphoxide in the bath did not exceed 0.4%. Such concentration did not alter airway smooth muscle responses to acetylcholine.

Expression of the results and statistical analysis

The effects of the relaxant agents were expressed as percentage of the relaxation produced by theophylline (THEO) 3.10^{-3}M. The contracturant response was

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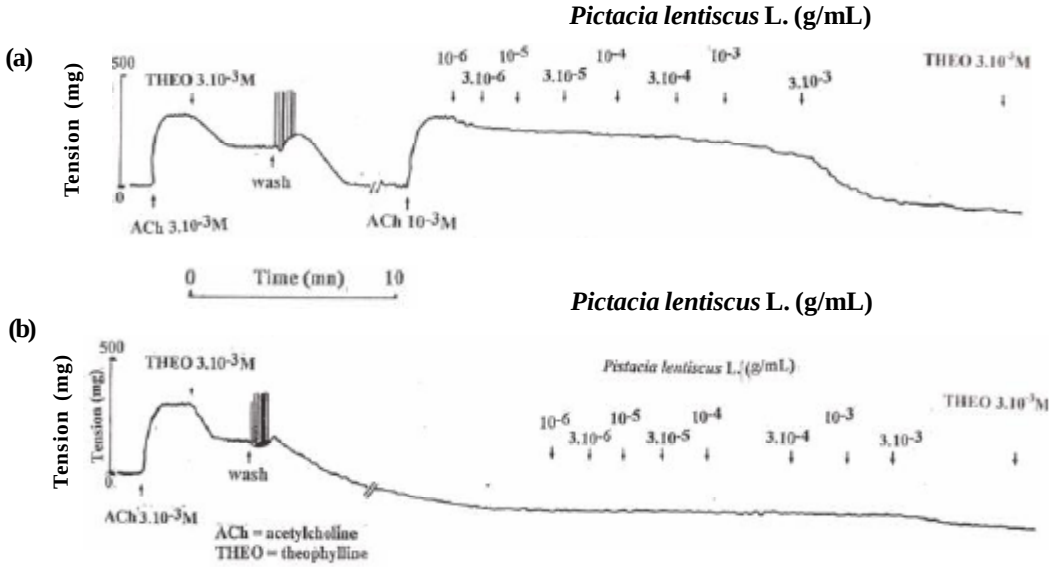

Figure 1 : Representative trace from the acetylcholine (ACh 10^{-3}M) (A) precontracted and the basal tone (B) rat isolated trachea showing cumulative dose-response to Pistacia lentiscus L. 70% ethanol extract. Concentrations are in g/mL. The last addition of the extract is followed by the addition of theophylline (3.10^{-3}M)

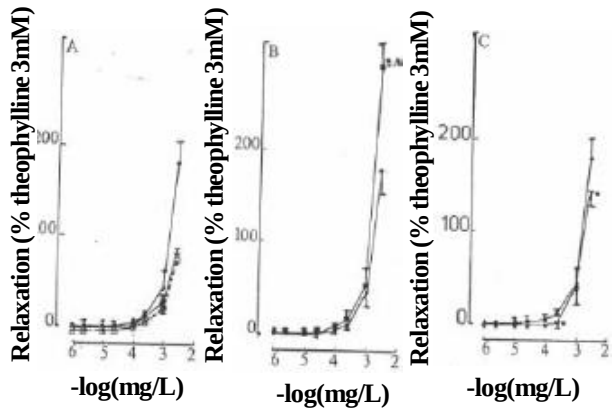

Figure 2 : Rat isolated trachea precontracted with acetycholine (Ach 10^{-3}M) A : Concentration-response curves for aqueous (---), 30% ethanol (o) and 70%(•) *Pictacia lentiscus* L. B: Concentration-response curves for *Pictacia lentiscus* L. 70 % ethanol extract in control (•) and after pretreatment with forskolin 10^{-6}M. C: Concentration-response curves for *Pictacia lentiscus* L. 70 % ethanol extract in control (•) and after pretreatment with methylene blue 3.10^{-5}M. Each point represents the mean value. S.e.m are shown by vertical bars. The results are expressed as percentage of maximal response Emax (theophylline 3.10^{-5}M). Significant differentces from control are shown as : * p<0.05 and ***p<0.001

expressed as a percentage of the contraction obtained with acetylcholine (ACh) 10^{-5}M. EC_{50} was defined as the concentration producing 50% of the maximal effect THEO 3.10^{-3}M or ACh 10^{-5}M. The negative logarithms of EC_{50} values (pD_2) were calculated from each concentration-response curve. All results were expressed as means S.E. mean and statistical significance was determined by Student's test.

RESULTS

Effect of *Pistacia lentiscus* L. extracts on the rat isolated trachea

Pistacia lentiscus L. extracts produced concentration-dependent relaxation on the rat tracheal preparation, as shown in figure 1. Relaxation occurred when the trachea was pre-contracted with ACh10^{-3}M and on resting tone trachea (Figure 1A and 1B).

EC_{50} values for the 70% ethanol, 30% ethanol and aqueous extracts were respectively as follows: $1.00.10^{-3} \pm 0.11$ (n=15), $1.71.10^{-3} \pm 0.09$ (n=8) and $1.75.10^{-3} \pm 0.12$ g/ml (n=12) (Figure 2 A).

Influence of atropine, propranolol, phentolamine, indomethacin, forskolin and methylene blue on the concentration-response curves to *Pistacia lentiscus* L. 70% ethanol extract.

In the presence of atropine (10^{-7}M), propranolol (10^{-7}M), phentolamine (10^{-7}M) indomethacin (10^{-6}M) and the direct acting G stimulant NaF, concentration-

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TABLE 1: Effect of pretreatment with atropine, propranolol, phentolamine, indomethacine, methylene blue, forskolin and NaF on relaxation of rat trachea to *Pistacia lentiscus* L. 70% ethanol extract

Pretreatment (M)	n	pD_2= -log EC_{50}	Emax/THEO 3.10^{-3}M
Control	15	2.98 ± 0.08	182.14 ± 17.52
Atropine 10^{-7}	6	2.92 ± 0.07	185.16 ± 9.19
Propranolol 10^{-7}	6	3.14 ± 0.19	179.84 ± 6.55
Phentolamine 10^{-7}	6	3.19 ± 0.09	183.24 ± 8.21
Indomethacin 10^{-6}	6	3.11 ± 0.15	182.76 ± 8.25
Methylene blue 3.10^{-5}	6	2.94 ± 0.04	143.14 ± 19.27*
Forskolin 10^{-6}	6	3.03 ± 0.04	308.62 ± 13.46***
NaF 10^{-4}	6	3.00 ± 0.01	192.44 ± 7.34

The preparations were precontracted with acetylcholine (ACh) 10^{-3}M; Their ability to relax the airway smooth muscle is expressed by Emax [maximal effect in relation to theophylline (THEO) 3.10^{-3}M] and by –log EC_{50} (concentration producing 50% of the maximal effect potency); Values are means ± s.e.m; n = number of experiments; Significant differences from control are shown as: * : p<0.05 and *** : p<0.001

response curves to *Pistacia lentiscus* L. were not modified (p<0.05) whereas, under the influence of forskolin 10^{-6}M, *Pistacia lentiscus* L. extract was significantly more efficacious (higher Emax) (TABLE 1 and Figure 2B). On the other hand, pretreatment with methylene blue (3.10^{-5}M) caused a right shift of *Pistacia lentiscus* L. concentration-response curves (Figure 2C).

Influence of *Pistacia lentiscus* L. 70% ethanol extract on the effect of captopril

Captopril induced on the rat isolated trachea precontracted with acetylcholine 10^{-5}M a concentration-dependent relaxation with EC_{50} value of $1.73.10^{-3}$ ± 0.34 M (n=7). In the presence of *Pistacia lentiscus* L. 70% ethanol extract (10^{-6} to 10^{-4} g/ml), concentration-response curves to captopril were significantly shifted to the left in a dose-dependent manner (Figure 3A).

Effect of *Pistacia lentiscus* L. 70% ethanol extract on the concentration-response curves to theophylline, IBMX, 8 phenyltheophylline, enprolylline, isoprenaline, nifedipine, dipyridamole and sodium nitroprusside

Theophylline, IBMX, 8 phenyltheophylline, enprolylline, isoprenaline, nifedipine, dipyridamole and sodium nitroprusside produced concentration-dependent relaxation of the rat tracheal preparations pre-contracted with ACh 10^{-5}M, as shown in table 2. The concentration-response curves to theophylline, IBMX,

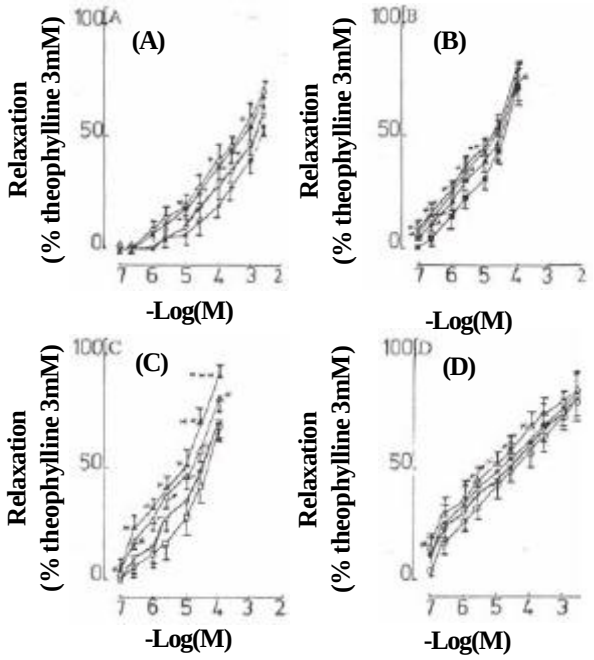

Figure 3 : Influence of *Pictacia lentiscus* L. 70% ethanol extract on the concentration-responce curves of A : Captopril concentration-effect curves in control (•), and after pretreatment with *Pictacia lentiscus* L. 70% ethanol extract 10^{-6}g/ml (∇) 10^{-5}g/ml (▲) 10^{-4}g/ml (Δ). B: 8 phenyltheophylline. Concentration-effect curves in control (■) and after pretreatment with *Pictacia lentiscus* L. 70 % ethanol extract 10^{-6}g/ml (∇), 10^{-5}g/ml (▲) 10^{-4}g/ml (Δ). C: Enprofylline. Concentration-effect curves in control (□) and after pretreatment with *Pictacia lentiscus* L. 70% ethanol extract 10^{-6}g/ml (∇) 10^{-5}g/ml (▲) 10^{-4}g/ml (Δ) . D: Sodium nitroprussiate. Concentration-effect curves in control (o) and after pretreatment with *Pictacia lentiscus* L. 70% ethanol extract 10^{-6}g/ml (∇), 10^{-5}g/ml (▲) 10^{-4}g/ml (Δ). preparations were contracted with acetylcholine (ACh 10^{-5}M). Each point represents the mean value. S.e.m. are shown by verticle bars. The results are expressed as precentageof maximal reponse Emax (theophylline 3.10^{-3}M) Significant differences from control are shown as:* p<0.05, ** p<0.01 and *** p<0.001

isoprenaline, nifedipine and dipyridamole were not affected by *Pistacia lentiscus* L. (10^{-6} to 10^{-4}g/ml) whereas, the relaxation of the rat trachea induced by 8 phenyltheophylline, enprofylline and sodium nitroprusside was significantly potentiated by the extract (10^{-6} to 10^{-4}g/ml), as indicated by the shift to the left of the concentration-response curves (TABLE 2 and Figures 3B, 3C and 3D).

Influence of *Pistacia lentiscus* L. 70% ethanol extract on the effects of specific nucleotide phospho

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TABLE 2 : Effect of pretreatment with *Pistacia lentiscus* L. 70% ethanol extract on relaxation of rat isolated trachea to theophylline, IBMX, 8 phenyltheophylline, enprofylline, isoprenaline, nifedipine, dipyridamole and sodium nitroprusside

Treatment	n	pD_2 = log EC_{50}	Emax/THEO 3.10^{-3}M
Theophylline			
Control	7	3.82 ± 0.08	
Pistacia lentiscus L.			
10^{-6} (g/ml)	6	3.79 ± 0.11	89.44 ± 2.08
10^{-5} (g/ml)	6	3.73 ± 0.19	92.77 ± 1.35
10^{-4} (g/ml)	6	3.62 ± 0.22	86.69 ± 4.14
IBMX			
Control	6	4.96 ± 0.09	88.52 ± 3.08
Pistacia lentiscus L.			
10^{-6} (g/ml)	6	4.57 ± 0.19	80.17 ± 8.76
10^{-5} (g/ml)	5	4.53 ± 0.12	85.28 ± 4.99
10^{-4} (g/ml)	6	4.83 ± 0.03	90.46 ± 3.22
8 PT			
Control	8	4.51 ± 0.08	71.63 ± 2.15
Pistacia lentiscus L.			
10^{-6} (g/ml)	6	4.55 ± 0.08*	73.40 ± 0.84*
10^{-5} (g/ml)	6	4.74 ± 0.03	76.93 ± 1.93*
10^{-4} (g/ml)	6	4.76 ± 0.05*	76.47 ± 0.29*
Enprofylline			
Control	7	4.46 ± 0.10	70.48 ± 3.81
Pistacia lentiscus L.			
10^{-6} (g/ml)	5	4.56± 0.13	70.88 ± 3.82
10^{-5} (g/ml)	6	4.91 ± 0.18*	80.36 ± 1.66*
10^{-4} (g/ml)	6	5.21± 0.22**	90.98± 2.48***
Isoprenaline			
Control	7	4.56 ± 0.13	72.54 ± 2.58
Pistacia lentiscus L.			
10^{-6} (g/ml)	6	4.62 ± 0.07	69.72 ± 4.89
10^{-5} (g/ml)	7	4.25 ± 0.14	75.58 ± 3.32
10^{-4} (g/ml)	6	4.34 ± 0.16	73.44 ± 2.65
Nifedipine			
Control	8	4.39 ± 0.15	97.79 ± 1.43
Pistacia lentiscus L.			
10^{-6} (g/ml)	6	4.75 ± 0.27	96.22 ± 1.31
10^{-5} (g/ml)	6	4.30 ± 0.11	96.19 ± 1.87
10^{-4} (g/ml)	6	4.55 ± 0.21	95.91 ± 0.92
Dipyridamole			
Control	8	4.15 ± 0.17	97.37 ± 1.10
Pistacia lentiscus L.			
10^{-6} (g/ml)	6	4.10 ± 0.22	95.41 ± 1.46
10^{-5} (g/ml)	6	3.87 ± 0.16	95.39 ± 1.43
10^{-4} (g/ml)	6	4.05 ± 0.22	98.72 ± 1.27
SNP			
Control	8	4.97 ± 0.13	80.01 ± 4.82
Pistacia lentiscus L.			
10^{-6} (g/ml)	7	4.89 ± 0.31	81.28 ± 3.76
10^{-5} (g/ml)	7	5.86 ± 0.33*	83.21 ± 3.47
10^{-4} (g/ml)	6	5.82 ± 0.37*	84.44 ± 3.42

The preparations were pr-contracted with acetylcholine 10^{-5}M. Their ability to relax the airway smooth muscle is expressed by Emax (maximal effect in relation to theophylline 3.10^{-3}M) and by –log EC50 (concentration producing 50% of the maximal effect); n is the number of experiments; Significant differences from control are shown as: *p<0.05; p<0.01; ***p<0.001; THEO: theophylline, IBMX: 3-isobutyl-1-methylxanthine, 8 PT: 8 phenyltheophylline, SNP: sodium nitroprussiate

diesterase (PDE) inhibitors

The effects of phosphodiesterase isoenzyme inhibitors on the rat isolated are summarized in figure 4A. Siguazodam produced on the rat isolated trachea precontracted with acetylcholine a dual effect characterized by a relaxation phase from 10^{-7} to 10^{-5}M followed by a contraction phase from 10^{-5} to 3.10^{-4}M. EC_{25} [concentration of agonist producing 25% of maximal effect (THEO 3.10^{-3}M)] and EC_{50} [concentration producing 50% of maximal effect (ACh 10^{-5}M)] values for relaxation and concentration were respectively of $1.38.10^{-3} \pm 0.42$ M and $140.43.10^{-3} \pm 47.59$ M (n=7).

Rolipram, zaprinast and sodium nitroprusside produced concentration-dependent relaxation of the rat isolated trachea with respectively EC_{50} values of $61.10.10^{-3} \pm 12.23$ (n = 7), $119.21.10^{-3} \pm 33.66$ (n = 7) and $17.74.10^{-3} \pm 5.62$ M (n = 8). Pistacia lentiscus L. did not modify concentration-effect curves to rolipram and siguazodam (Figures 4B and 4C) whereas, pretreatment with the plant extract produced complete reversal of the zaprinast-induced relaxation. This reversal response occurred from 10^{-4}M zaprinast concentration added to the bath (Figure 4 D).

Effect of *Pistacia lentiscus* L. 70% ethanol extract on adenosine

On pre-contracted rat trachea with acetylcholine 10^{-5}M, adenosine caused contraction in lower concentrations (10^{-6} to 10^{-4}M) and relaxation in higher concentrations (3.10^{-4} to 3.10^{-3}M) (Figure 5A). The contractile response to adenosine was 16.97 ± 2.93% (n=11) of the response to ACh 10^{-5}M. The relaxation to adenosine was 82.57 ± 4.45% (n=11) of the response to THEO 3.10^{-3}M and EC_{50} of adenosine-induced relaxation is $1.43.10^{-3} \pm 022$ M.

Pistacia lentiscus L. 70% ethanol extract (10^{-6} to 10^{-4} g/ml) abolished contractions to adenosine. Furthermore, in the presence of the plant extract, adenosine-induced relaxation was potentiated. This reflected a decrease in the EC50 values from $1.43.10^{-3} \pm 022$ M for the control group to $0.74.10^{-3} \pm 0.17$ M ($p<0.05$) (n=6), $0.48.10^{-3} \pm 0.11$ M ($p<0.01$) and $0.28.10^{-3} \pm 0.05$ M ($p<0.001$) (n=6) values respectively obtained in the presence of 10^{-6}, 10^{-5} and 10^{-4} g/ml *Pistacia lentiscus* L. extract (Figure 5 B). The effect of *Pistacia lentiscus* L. on adenosine dose-response curves was

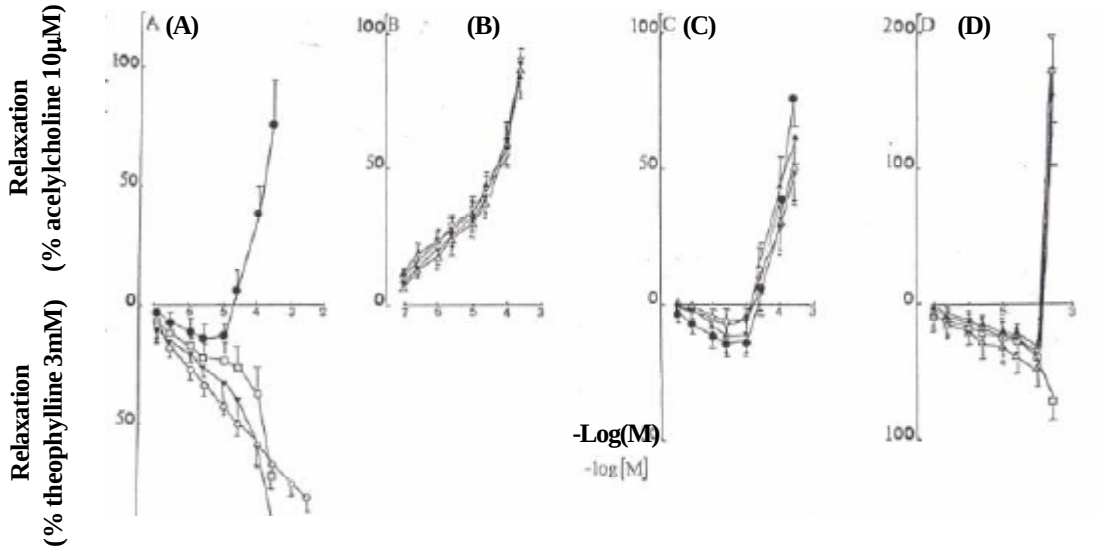

Figure 4: Concentration -effect curves in for rolipram(▼), siguazodam (•), zaprinast(□) and sodium nitroprusside (o) on the rat isolated trachea. B: rolipram concentration-effetc curves in control (▼) and after pretreatment with *Pictacia lentiscus* L. 70% ethanol extract 10^{-6}g/ml (∇) 10^{-5}g/ml (▲) 10^{-4}g/ml (Δ). C: Siguazodam: Concentration-effetc curves in control(•) and after pretreatment with *Pictacia lentiscus* L. 70% ethanol extract 10^{-6}g/ml (∇) 10^{-5}g/ml (▲) 10^{-4}g/ml (Δ). D: Zaprinast Concentration-effetc curves in control(□) and after pretreatment with *Pictacia lentiscus* L. 70% ethanol extract 10^{-6}g/ml (∇) 10^{-5}g/ml (▲) 10^{-4}g/ml (Δ). Preparations were contracted with acetylcholine (ACh 10^{-5}M). Each point represents the mean value. S.e.m. are shown by vertical bars. The results are expressed as precentage of maximal reponse Emax (theophylline 3.10^{-3}M) for relaxation and Emax (acetylcholine 10^{-5}M)for contraction

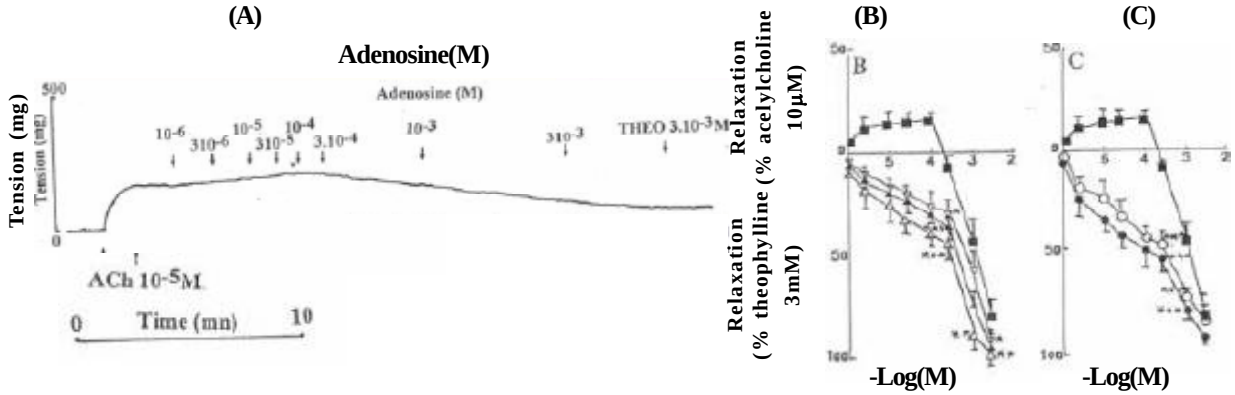

Figure 5: A: Representative trace of the acetylcholine (ACh 10^{-5}M) precontracted rat isolated trachea showing cumulative dose-response to adenosine. Concentrations are shown in M. B: Influence of *Pictacia lentiscus* L. 70% ethanol on the concentration-response curves of adenosine. Concentration-effect curves in control (■) and after pretreatment with *Pictacia lentiscus* L. 70% ethanol extract 10^{-6}g/ml (∇) 10^{-5}g/ml (▲) 10^{-4}g/ml (Δ). C: Influence of 8 phenytheophylline on the concentration-response curves of adenosine. Concentration-effect curves in control (■) and after pretreatment with 8 phenytheophyllin 10^{-8}M (•) and 10^{-7}M (o). Preparations were contracted with acetylcholine (ACh 10^{-5}M). Each point represents the mean value. S.e.m. are shown by vertical bars.The results are expressed for relaxation and Emax (acetylcholine 10^{-5}M) for relaxation. Significant differences from control are shown as: *p<0.05, **p<0.01 and ***p<0.001

similar to the one induced by 8 phenyltheophylline 10^{-8} and 10^{-7} M (Figure 5B).

DISCUSSION AND CONCLUSION

The main finding of this work is that *Pistacia*

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lentiscus L. extracts relax the rat trachea under basal tone and after pre-contraction with acetylcholine 10^{-3}M. The 70% ethanol extract was more potent than the 30% ethanol and aqueous extracts. This finding suggests that a more lipophilic compound may possess greater activity than the water soluble extract.

Direct interference with α-adrenoceptors, β-adrenoceptors and cholinergic receptors can be excluded since *Pisticia lentiscus* L. induced relaxation was not respectively modified by phentolamine, propranolol and atropine. Moreover, this effect is not mediated by arachidonic acid derivates produced under the influence of cyclo-oxygenase since concentration-response curves to *Pistacia lentiscus* L. were not modified by indomethacin.

Pistacia lentiscus L. 70% ethanol extract (10^{-6} to 10^{-4}g/ml) significantly potentiated captopril-induced relaxation in a dose-dependent manner. These data may suggest that the studied drug produces an inhibition of ACE. These results are in agreement with those of Sanz et al.,[23,24] who suggested a possible involvement of the angiotensin system as the mechanism of action of *Pistacia lentiscus* L.

Though an intervention on 3′5′-cyclic monophosphate (cyclic AMP) system cannot be excluded since the adenylcyclase stimulator forskolin[10] potentiated *Pistacia lentiscus* L. induced relaxation, many evidences may suggest this latter could also result from a mechanism of action involving an inhibition of ACE.

In airway smooth muscle two components have been demonstrated in the non-adrenergic non-cholinergic (NANC) nerves[5,17]. An inhibitory (i-NANC) pathway which may be mediated by nitric oxide (NO)[30] and an excitatory (e-NANC) mediated tachykinins (substance P, neurokinin A)[17].

The excitatory NANC nerves are activated by mediators such as bradykinin, prostaglandins, substance P, calcitonine gene-related peptide (CGRP)[5]. The release of these neuropeptides will lead to NANC bronchoconstriction. These neuropeptides are degraded by a neutral endopeptidase (enkephalinease) and the angiotensin converting enzyme (ACE)[7]. Inhibition of ACE will result to an increase in the activity of e-NANC nerves. Inhibition of ACE induced by *Pistacia lentiscus* L. would by this way conduct to an increase of the e-NANC activity. Since a balance exists between the two components of the NANC system, the consequence would be an activation of the i-NANC system in order to counteract the e-NANC stimulation. Activation of the i-NANC system would be responsible of the recorded relaxation induced by the plant. One observation supports this suggestion: methylene blue, a substance that inhibits the effects of sodium nitroprusside[22] and the activation of guanulate cyclase to its soluble form by NO donating drugs[13], antagonized *Pistacia lentiscus* L.-induced relaxation. Since nitric oxide (NO) is an i-NANC neurotransmitter, it is therefore possible to suggest that the recorded relaxation could be due to an activation of this system.

Since the adenylcyclase stimulator forskolin[10] potentiated *Pistacia lentiscus* L. induced relaxation, it has been decided to study the effect of the drug on agents that enhance c-AMP or c-GMP by inhibition of phosphodiesterase (PDE).

Pistacia lentiscus L. did not modify concentration-response curves to theophylline and IBMX, two non selective PDE inhibitors[19]. Such observation was also obtained with nifedipine and dipyridamole, two compounds behaving PDE inhibitor activities[28].

We also investigated the effect of the drug on isoenzyme selective inhibitors. The inhibitors were: siguazodan, a PDE III inhibitor[28], rolipram, a PDE IV selective inhibitor[21] and zaprinast, a PDE V selective inhibitor[34].

Concentration-response curves to rolipram and siguazodam were not significantly modified in the presence of *Pistacia lenticus* L. This excludes an involvement of an inhibition of PDE III or IV in the recorded relaxation. In the opposite, many evidence allows to suggest that type V isoenzyme that hydrolyses c-GMP (guanosine 3'5'-cyclic monophosphate)[27] does interfere in *Pistacia lentiscus* L.-induced relaxation since, in the presence of the plant extract, total reversal of the zaprinast-induced relaxation was shown from 10^{-4}M agonist concentration. Our conclusion is also supported by the fact that *Pistacia lentiscus* L. extract potentiated in a dose-dependent manner the effect of sodium nitroprusside, a guanylate cyclase activator that is thought to relax airway smooth muscle via c-GMP mediated mechanism[28].

Finally, we studied the effect of *Pistacia lentiscus* L. on 8 phenyltheophylline and enprofylline-induced relaxations. The results showed that *Pistacia lentiscus* L. potentiated the effects of these two xanthines. Since

8 phenyltheophylline is a potent antagonist at adenosine receptors[26], it was decided to investigate the effect of *Pistacia lentiscus* L. on adenosine action.

On precontracted rat trachea, adenosine was shown to cause contractile response and predominant relaxation. According to Burnstock[8] classification, the contraction and the relaxation are respectively mediated by A_1 and A_2 purinergic receptors activation. *Pistacia lentiscus* L. blocked adenosine contractile response to about the same extent as the A_1 receptor antagonist 8 phenyltheophylline. Furthermore, relaxation induced by the purine nucleoside was potentiated in the presence of the plant extract. It is likely that this potention is mediated by an activation of the adenylate cyclase system through A_2 receptors stimulation since forskolin, a substance known to directly stimulate adenylcyclase[14] increased *Pistacia lentiscus* L.-induced relaxation. The mechanism of action by which the plant extract potentiated enprofylline-induced relaxation is less evident to establish since this anti-asthmatic drug is described to be without adenosine antagonistic properties[20]. However, we cannot exclude a mechanism of action at this level since enprofylline was reported to exhibit adenosine receptor antagonistic properties[31,33].

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