# Cardiovascular Effects Induced by Linalool in Normotensive and Hypertensive Rats

Paulo J. C. Anjos[a], Aline O. Lima[a], Patrícia S. Cunha[a], Damião P. De Sousa[a], Alexandre S. C. Onofre[a], Thais P. Ribeiro[b], Isac A. Medeiros[b], Ângelo R. Antoniolli[a], Lucindo J. Quintans-Júnior[a], and Márcio R. V. Santos[a,*]

[a] Department of Physiology, Federal University of Sergipe, São Cristóvão – SE, Brazil. Fax: (+55) 79 2105-6474. E-mail: marcio@infonet.com.br

[b] Laboratory of Pharmaceutical Technology (LTF), Federal University of Paraíba, João Pessoa – PB, Brazil

* Author for correspondence and reprint requests

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Linalool is a monoterpene alcohol and constituent of several Brazilian aromatic medicinal plants, popularly used against hypertension. Cardiovascular effects induced by linalool were evaluated. In normotensive rats, (±)-linalool [1, 5, 10, and 20 mg/kg body weight (BW); intravenous (i.v.)]-induced hypotension was associated with tachycardia, which was attenuated by atropine (2 mg/kg BW) and $N^G$-nitro-L-arginine methyl ester (20 mg/kg BW), but was not modified after indomethacin (5 mg/kg BW) administration. In hypertensive rats, linalool [200 mg/kg BW; oral (v.o.)] reduced blood pressure without changing the heart rate. In intact rings of rat mesenteric artery precontracted with 10 $\mu$M phenylephrine, linalool (from $6.4 \cdot 10^{-6}$ to $6.4 \cdot 10^{-3}$ M) induced relaxations in a concentration-dependent manner [$E_{max}$ = (115 ± 13)%] that were not changed after atropine administration [$E_{max}$ = (105 ± 2)%], and were not different from those obtained in endothelium-denuded rings precontracted with phenylephrine [$E_{max}$ = (108 ± 7)%] or 80 mM KCl [$E_{max}$ = (113 ± 7)%] or tetraethylammonium incubation [$E_{max}$ = (105 ± 12)%]. Linalool ($1.9 \cdot 10^{-3}$ M) antagonized the contractions induced by $CaCl_2$ ($3 \cdot 10^{-6}$–$10^{-2}$ M) (maximal inhibition, 81%). Furthermore, linalool inhibited the contractions induced by 10 $\mu$M phenylephrine or 20 mM caffeine. In conclusion, these results demonstrate that linalool reduces blood pressure probably due to a direct effect on the vascular smooth muscle leading to vasodilation.

*Key words:* Linalool, Arterial Pressure, Vascular Smooth Muscle

## Introduction

Essential oils are natural, complex, multi-component systems composed mainly of terpenes in addition to some other non-terpene components (Edris, 2007). These volatile substances are commonly found in aromatic plants, and their therapeutic potential has been widely evaluated (Edris, 2007; Kris-Etherton *et al*., 2002; Paduch *et al*., 2007). Studies in humans (Paduch *et al*., 2007; Shiina *et al*., 2008; Dayawansa *et al*., 2003) and animals (De Sousa *et al*., 2006) have demonstrated beneficial properties of essential oils in the cardiovascular system, such as antithrombotic, antiplatelet, endothelial protective, vasorelaxant, hypotensive, bradicardic activities (Edris, 2007; Shiina *et al*., 2008; Dayawansa *et al*., 2003), and improvement in coronary flow (Shiina *et al*., 2008).

The use of medicinal plants as an alternative to conventional medicine in the treatment of cardiovascular diseases has increased considerably worldwide (Cirigliano and Sun, 1998). Because of this trend, in many reports the effects of several medicinal plants and their constituents on the cardiovascular system have been evaluated, aiming to provide a scientific basis for the therapeutic applications (Menezes *et al*., 2007; Santos *et al*., 2007). In this context, essential oils extracted from medicinal plants have been studied, and their therapeutic potential has been demonstrated in animals (De Sousa *et al*., 2006).

Linalool (Fig. 1), an open-chain monoterpene tertiary alcohol, is found naturally as a racemic mixture (De Sousa *et al*., 2010). It is present in several aromatic medicinal plants as major constituent of the essential oils (Linck *et al*., 2009). Several of its pharmacological activities have

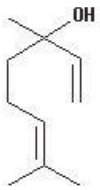


Fig. 1. Chemical structure of linalool.

been studied, such as sedative (Linck *et al*., 2009), anxiolytic (Linck *et al*., 2010), and anticonvulsant (De Sousa *et al*., 2010; Brum *et al*., 2001). Studies demonstrated that linalool does not present significant toxicological and mutagenic effects (Bickers *et al*., 2003; Di Sotto *et al*., 2008). Previous results obtained in our laboratory demonstrated that linalool induces hypotension in normotensive rats, however, the mechanism of its action was not investigated further (Menezes *et al*., 2010).

Although linalool is widely used in pharmaceutical and cosmetic industries (Zheljazkov *et al*., 2008) and is a constituent of Brazilian aromatic medicinal plants popularly used against hypertension (Hennebelle *et al*., 2008; Tavares *et al*., 2005), no study reporting its action on the cardiovascular system could be found in the literature. Thus, the objective of the present work was to evaluate the antihypertensive activity of linalool using *in vitro* and *in vivo* approaches.

## Material and Methods

### *Drugs*

The drugs used were: (*RS*)-(±)-linalool (purity, 97%) (Dierberger, Barra Bonita, SP, Brazil); sodium thiopental (Cristalia, Itapira, SP, Brazil); heparin (Roche, São Paulo, SP, Brazil); atropine sulfate, $N^G$-nitro-L-arginine methyl ester (L-NAME), indomethacin (INDO), nifedipine (NIF), L-phenylephrine chloride (Phe), acetylcholine chloride (Ach), tetraethylammonium chloride (TEA), oxytetracyclin chloride, and caffeine (CAF) (all from Sigma Chemical Co, St. Louis, MO, USA). In the preparation of the stock solutions, linalool was diluted in Tyrode's/cremophor solution (0.15%, v/v) for *in vitro* experiments, or saline/cremophor solution (0.15%, v/v) for *in vivo* experiments. INDO was dissolved together with sodium bicarbonate ($NaHCO_3$) to 5% in distilled water, and the other drugs were dissolved in distilled water only. All stock solutions were maintained at 0 °C and diluted to the desired concentration with distilled water or saline, when necessary. Cremophor revealed no effect in control experiments.

### *Solutions*

The composition of the normal Tyrode's solution was (in mM): NaCl (158.3), KCl (4.0), $CaCl_2$ (2.0), $NaHCO_3$ (10.0), $MgCl_2$ (1.05), $NaH_2PO_4$ (0.42), and D-glucose (5.6). The $K^+$-depolarizing solutions (80 and 60 mM KCl) were prepared by replacing 80 or 60 mM KCl in Tyrode's solution with equimolar NaCl. In a solution nominally without $Ca^{2+}$, $CaCl_2$ was omitted.

### *Animals*

Sixty six male Wistar normotensive rats (160–300 g) were obtained from colonies maintained in the Department of Physiology, Federal University of Sergipe, São Cristóvão, SE, Brazil, and in the George Thomas Bioterium, Federal University of Paraíba, João Pessoa, PB, Brazil. They were maintained in a large cage under controlled conditions of temperature and lighting (light on, 06:00 a.m.–06:00 p.m.), fed with rodent diet and tap water *ad libitum*. All procedures had been approved by the Animal Research Ethics Committee of the Federal University of Sergipe and Federal University of Paraíba (protocol numbers 16/08 and 0102/10, respectively) and were in compliance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication 85–23, revised 1996).

### *Effects of linalool on blood pressure and heart rate in normotensive conscious rats*

Thirty six normotensive rats (200–300 g) were anaesthetized with sodium thiopental [45 mg/kg body weight (BW); intraperitoneal (i.p.) injection], and polyethylene catheters were implanted in the abdominal aorta and inferior vein cava via the artery and left femoral vein. After insertion and fixation by cotton threads, the catheters were tunneled subcutaneously and exteriorized through an incision in the posterior cervical region of the animal. The incisions were sutured, and the animals were given a postoperative period of 24 h.

After the stabilization period, mean arterial pressure (MAP) and heart rate (HR) were recorded before (baseline values) and after *in bolus*

administration of linalool [1, 5, 10, and 20 mg/kg BW; intravenous (i.v.)] to obtain the control values for the dose-response curve. Similar records with linalool were obtained after treatment with atropine (2 mg/kg BW; i.v.; 30 min), L-NAME (20 mg/kg BW; i.v.; 30 min), or INDO (5 mg/kg BW; i.v.; 30 min), separately. These curves ($n$ = 6, each) were compared with the control curve ($n$ = 6).

*Effects of linalool on blood pressure and heart rate in Goldblatt hypertensive conscious rats*

Two-kidney one-clip Goldblatt hypertension was induced in normotensive rats (160–180 g) by the method of Goldblatt *et al.* (1934) as adapted by Schaffenburg (1959). The animals were anaesthetized with sodium thiopental (45 mg/kg BW; i.p.) and submitted to median laparotomy, which exposed the renal pediculum. Afterwards, one silver clip (2 mm x 5 mm, 0.2 mm ID) was placed around the left renal artery. After incision suture, oxytetracyclin chloride was administered in the animals in a single dose (0.2 g/kg BW), by the intramuscular way.

Hypertension (MAP > 160 mm Hg) was evidenced by direct measurements of MAP (as described above) 30 d after the surgical procedure. After this, eighteen hypertensive animals were acutely treated orally (via gavage) with a single dose of 10 ml/kg BW or with linalool (200 mg/kg BW; $n$ = 6), NIF (3 mg/kg BW; $n$ = 6), a drug reference, or with vehicle (saline + cremophor; $n$ = 6; control hypertensive animals). The measurements of MAP and HR were taken before and 0.5, 1, 2, 3, 4, 8, and 24 h after the treatments. The results were compared between groups.

*Tissue preparation*

The tissue preparation was performed as described by Menezes *et al.* (2007). Rats were killed by exsanguination under diethyl ether anaesthesia, and the superior mesenteric artery was removed, cleaned from connective and fat tissues, and sectioned in rings (1–2 mm). These rings were suspended in organ baths containing 10 ml of Tyrode's solution, gassed with carbogen, and maintained at 37 °C under a resting tension of 0.75 g for 60 min (stabilization period). The isometric tension was recorded by a force transducer (Model TRI210; Letica, Barcelona, Spain) coupled to an amplifier recorder. When necessary, endothelium was removed, and its functionality was assessed by the ability of Ach (10 $\mu$M) to induce more than 70% relaxation of Phe (10 $\mu$M) tonus. The absence of the relaxation for Ach was taken as evidence that the rings were functionally denuded of endothelium.

*Effects of linalool on Phe (10 $\mu$M) or KCl (80 mM) tonus in rings with and without endothelium*

Contractions of the vessels were induced with 10 $\mu$M Phe or 80 mM KCl in rings with or without endothelium. During the tonic phase of the contraction, linalool ($6.4 \cdot 10^{-6}$, $1.9 \cdot 10^{-5}$, $6.4 \cdot 10^{-5}$, $1.9 \cdot 10^{-4}$, $6.4 \cdot 10^{-4}$, $1.9 \cdot 10^{-3}$, and $6.4 \cdot 10^{-3}$ M, cumulatively) was added to the organ bath. The extent of relaxation was expressed as the percentage of Phe- or KCl-induced contraction. Furthermore, curves for linalool were obtained before and after incubation with 0.01 $\mu$M atropine, in rings with endothelium, or 1 mM TEA in rings without endothelium.

*Effects of linalool on $CaCl_2$ contractions in endothelium-denuded rings*

The effects of linalool on $CaCl_2$ contractions in endothelium-denuded rings were assessed using a protocol described by Santos *et al.* (2007). Cumulative concentration-response curves for $CaCl_2$ ($3 \cdot 10^{-6}$–$10^{-2}$ M) were obtained in rings without endothelium exposed to a solution nominally without $Ca^{2+}$ with 60 mM KCl before and after individual preincubation with linalool ($1.9 \cdot 10^{-4}$, $6.4 \cdot 10^{-4}$, and $1.9 \cdot 10^{-3}$ M) for 15 min. The results were expressed as percentages of the maximal response for $CaCl_2$ alone, and the curves were compared.

*Effects of linalool on Phe- and CAF-induced contractions in $Ca^{2+}$-free solution*

The effects of linalool on Phe- or CAF-sensitive intracellular calcium stores were assessed using a protocol described by Sakata and Karaki (1991) and Adaramoye *et al.* (2009). The transient contractions ($n$ = 6) were obtained in endothelium-denuded rings by 10 $\mu$M Phe or 20 mM CAF in $Ca^{2+}$-free solution before and after incubation with linalool ($6.4 \cdot 10^{-6}$–$6.4 \cdot 10^{-3}$ M) for 20 min. The results were expressed as percentages of the response induced by Phe or CAF alone.

*Statistic analysis*

Values were expressed as means ± standard error of the mean (S.E.M.). The results were analysed by one- or two-way ANOVA followed by Bonferroni post-test or paired or unpaired Student´s t test. The p$D_2$ values of *in vitro* experiments were obtained by non-linear regression. All procedures were performed using Graph Pad Prism 3.02™.

## Results

*Effects of linalool on hemodynamic parameters in normotensive conscious rats*

In normotensive conscious rats ($n = 6$), baseline MAP and HR values were (116 ± 4) mmHg and (376 ± 13) beats per minute (bpm), respectively. In these animals, intravenous bolus injections of linalool (1, 5, 10, and 20 mg/kg BW) induced hypotension and tachycardia (Figs. 2 and 3A). The administration of atropine produced a significant and sustained increase in the baseline value of HR [from (376 ± 13) to (412 ± 9) bpm], but did not alter MAP. However, L-NAME administration was able to significantly increase the baseline value of MAP [from (116 ± 4) to (156 ± 7) mmHg], but did not interfere with HR. After INDO administration, no change in MAP or HR baseline values was observed. As demonstrated in Fig. 3B, the hypotensive response induced by linalool was significantly altered by atropine in doses of 1, 5, and 10 mg/kg BW [(–3 ± 2)%, (–2 ± 3)%, (–12 ± 2)%, respectively; $n = 6$], while the tachycardic response was abolished at all doses [(0 ± 0)%, (0 ± 0)%, (–3 ± 1)%, (2 ± 3)%, respectively; $n = 6$]. In the presence of L-NAME, the hypotensive response induced by linalool was significantly reduced at doses of 1 and 5 mg/kg BW [(–3 ± 1)% and (–8 ± 3)%; $n = 6$], while the tachycardic response was not changed. Finally, after pretreatment with INDO, any parameter was modified (Fig. 3B).

*Effects of linalool on blood pressure and heart rate in Goldblatt hypertensive conscious rats*

In Goldblatt hypertensive conscious rats, the values of MAP and HR were (182 ± 6) mmHg and (429 ± 15) bpm, respectively. In these animals, acute oral administration of NIF was able to significantly reduce MAP without HR alteration, achieving the maximum effect 1 h after administration, with a decline of (46 ± 11)% (Fig. 4). The same way, linalool (200 mg/kg BW) was able to significantly reduce MAP without HR alteration, achieving the maximum effect 3 h after administration, with a decline of (25 ± 8)% (Fig. 4).

*Effects of linalool on Phe or KCl tonus in rings with and without endothelium, or after atropine or TEA incubation*

In rings of rat mesenteric artery with functional endothelium (control) precontracted with 10 $\mu$M Phe, linalool induced relaxations [p$D_2$ = (2.5 ± 0.2)%; $E_{max}$ = (115 ± 13)%; $n = 6$] that were not changed after removal of endothelium [p$D_2$ = (2.4 ± 0.1)%; $E_{max}$ = (108 ± 7)%; $n = 6$] or after atropine administration [p$D_2$ = (2.5 ± 0.1)%; $E_{max}$ = (105 ± 2)%; $n = 6$] (Fig. 5A). In rings without functional endothelium and pre-contracted with 80 mM KCl, linalool was able to induce relaxations [p$D_2$ = (2.0 ± 0.1)%; $E_{max}$ = (113 ± 7)%; $n = 6$] (Fig. 5B) that were not significantly different from those obtained in rings without functional endothelium and precontracted with Phe (10 $\mu$M). Furthemore, TEA (1 mM) did not affect linalool-induced relaxations [p$D_2$ = (2.0 ± 0.3)%; $E_{max}$ = (105 ± 12)%; $n = 6$] in rings without endothelium precontracted with Phe (Fig. 5B).

*Effects of linalool on $CaCl_2$-induced contractions in endothelium-denuded rings*

The incubation with $1.9 \cdot 10^{-3}$ M linalool antagonized the contractions induced by $CaCl_2$ ($3 \cdot 10^{-6}$–$10^{-2}$ M) (Fig. 6).

*Effects of linalool on Phe- and CAF-induced contractions in $Ca^{2+}$-free solution*

In mesenteric rings in $Ca^{2+}$-free solution, linalool inhibited transient contractions induced by 10 $\mu$M Phe (maximal response, 70.66%) or by 20 mM CAF (maximal response, 72.46%) (Fig. 7).

## Discussion

In Brazil, many hypertensive patients with associated cardiovascular diseases drink daily tea prepared from medicinal plants containing linalool (Zheljazkov *et al.*, 2008). This study demonstrates possible beneficial effects of linalool on the cardiovascular system. Our results demonstrate that linalool appears to have a calcium-blocking prop-

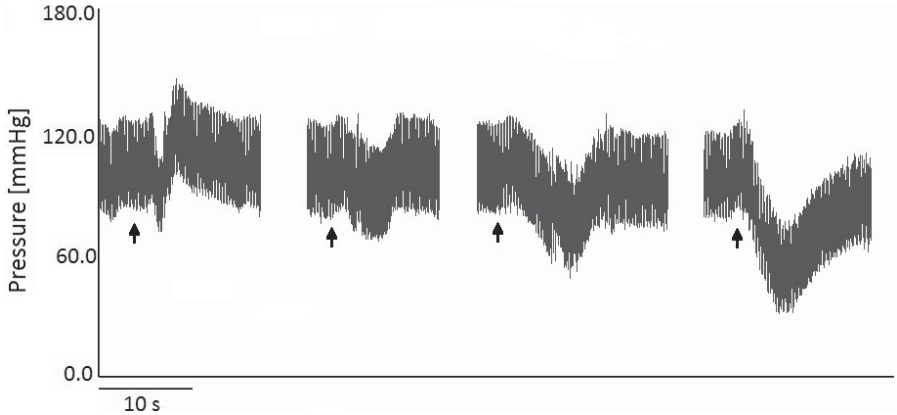


Fig. 2. Original records of the linalool effect on the arterial pressure of one control normotensive rat. Arrows indicate the time of administration.

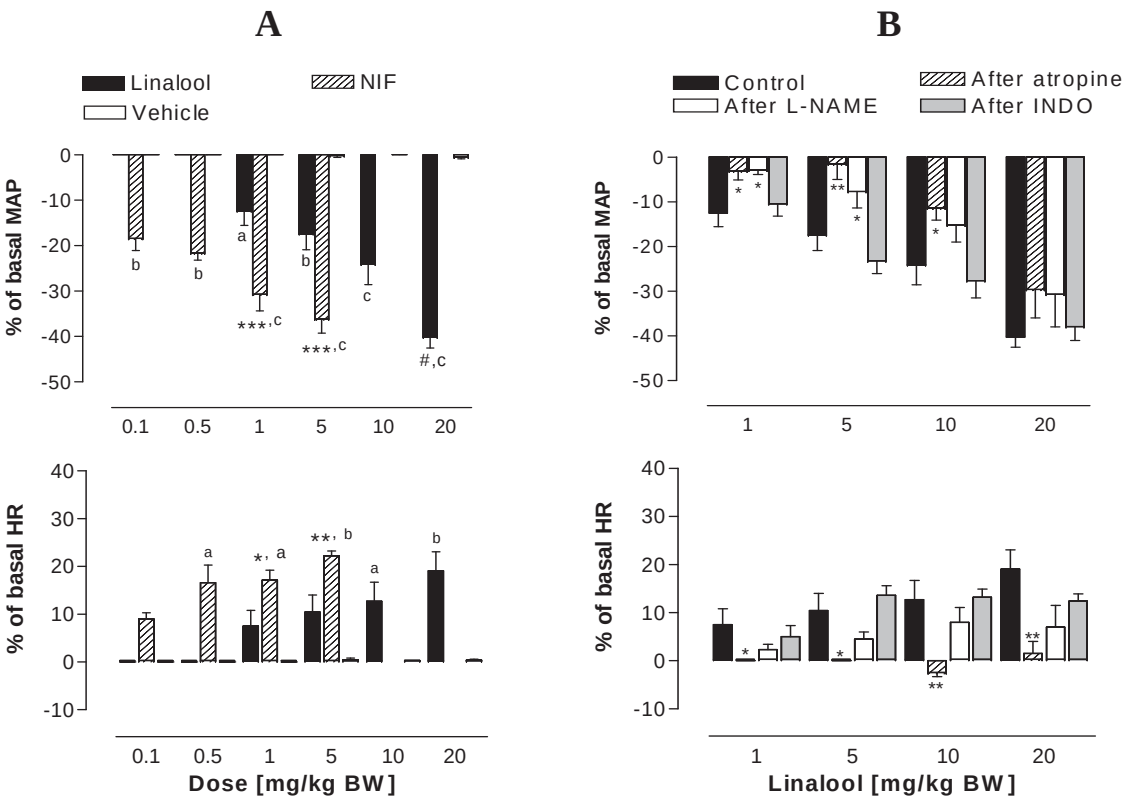


Fig. 3. Effect of linalool on the mean arterial pressure (MAP) and heart rate (HR) in normotensive conscious rats: (A) effect of linalool, nifedipine (NIF), and vehicle (saline + cremophor); (B) effect of linalool in rats before (control) and after pretreatment with atropine (2 mg/kg BW; i.v.), L-NAME (20 mg/kg BW; i.v.), and indomethacin (INDO) (5 mg/kg BW; i.v.). The data are expressed as means ± S.E.M. of 6 experiments for each protocol. To evaluate statistic differences between dose effects and basal MAP, one-way ANOVA was used followed by Bonferroni post-test. $^{a}p < 0.05$, $^{b}p < 0.01$, or $^{c}p < 0.001$ vs. basal MAP; $^{\#}p < 0.05$ vs. 10 mg/kg BW. To evaluate differences between groups, repeated measures two-way ANOVA was used followed by Bonferroni post-test. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ vs. linalool.

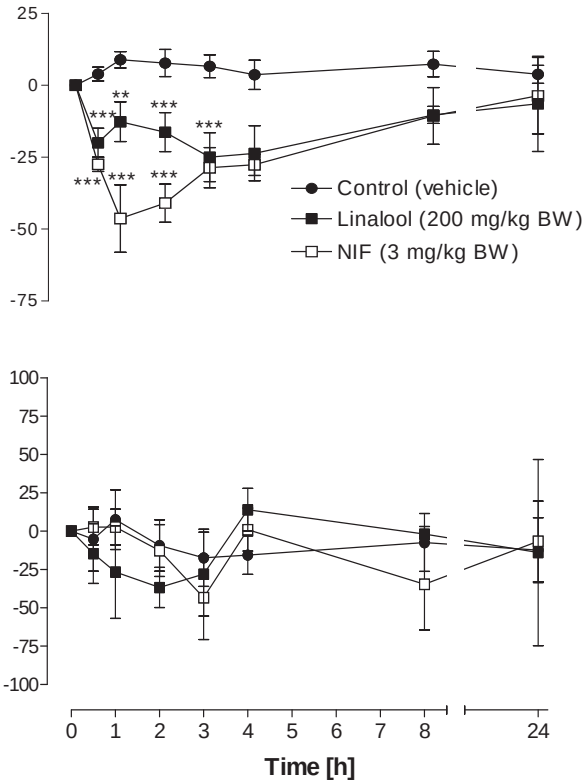


Fig. 4. Effect of oral administration of linalool (200 mg/kg BW) on the mean arterial pressure (MAP) and heart rate (HR) in Goldblatt hypertensive conscious rats before (0) and after 0.5, 1, 2, 3, 4, 8, and 24 h of treatment. Values are expressed as means ± S.E.M. of 6 experiments. $^{**}p < 0.01$ and $^{***}p < 0.001$ vs. control.

erty as is the case for many drugs used in the treatment of hypertension, such as amilodipine, NIF, and verapamil (Sociedade Brasileira de Cardiologia, 2007). Because of its vasorelaxant activity, linalool could be used as a potential substance for antihypertensive treatment.

Our results demonstrate that in normotensive conscious and freely moving rats, the intravenous administration of linalool induces intense hypotension associated with tachycardia. Also, linalool produced a vasorelaxant effect in the rat mesenteric artery by an endothelium-independent mechanism.

It is known that in normotensive animals, the vascular tone of the arterial bed underlies the maintenance of peripheral resistance in the circulation and it is the major contributor to the control of blood pressure (White *et al*., 1996). Furthermore, in most vascular beds, the activation of muscarinic receptors in the endothelial cells induces vasorelaxation by the release of endothelium-derived relaxant factors (EDRFs), including nitric oxide (NO) and cyclooxygenase (COX) metabolites, such as prostacyclin ($PGI_2$) (Moncada *et al*., 1991). In animals pretreated with atropine, a non-selective antagonist of these receptors (Mitchelson, 1984), the hypotensive and tachycardic responses are significantly changed, suggesting that linalool acts via muscarinic receptor activation. It is known that drugs that induce hypotension by reducing the pheripheral vascular resistance, such as NIF, are also able to cause a reflex tachycardia via the baroreflex system (Sociedade Brasileira de Cardiologia, 2007). The inhibition of tachycardia by atropine does not appear to be a direct action of atropine, but rather to be caused by two factors: decrease of the hypotensive effect and an intense positive inotropic response of the atropine treatment. Since the heart rate strongly increased, the tachycardic response induced by the indirect action of linalool (baroreflex response) was attenuated. Both events possibly reduced the baroreflex response.

L-NAME, an inhibitor of NO synthase (Moncada *et al*., 1991), was able to change the response induced by linalool in doses of 1 and 5 mg/kg BW, suggesting that, at least in part, NO may participate in this effect. On the other hand, the treatment with INDO, a potent non-selective COX inhibitor (Clark and Fuchs, 1997), did not significantly change the effects induced by linalool, suggesting that $PGI_2$ does not participate in this effect.

In Goldblatt hypertensive conscious rats, linalool was able to induce an antihypertensive effect with a magnitude similar to that produced by NIF. These results demonstrate that orally administered linalool has an antihypertensive effect, which can provide a scientific basis for a possible use of medicinal plants containing linalool in the management of arterial hypertension.

A decrease in blood pressure in hypertensive rats induced by administration of essential oils, such as *Mentha* x *villosa* and *Ocimum gratissimum* essential oils, was also related by Lahlou *et al.* (2002a) and Interaminense *et al*. (2005). These results, together with those from the literature, strengthen the importance of studies with essential oils in the search for new drugs for the treatment of hypertension.

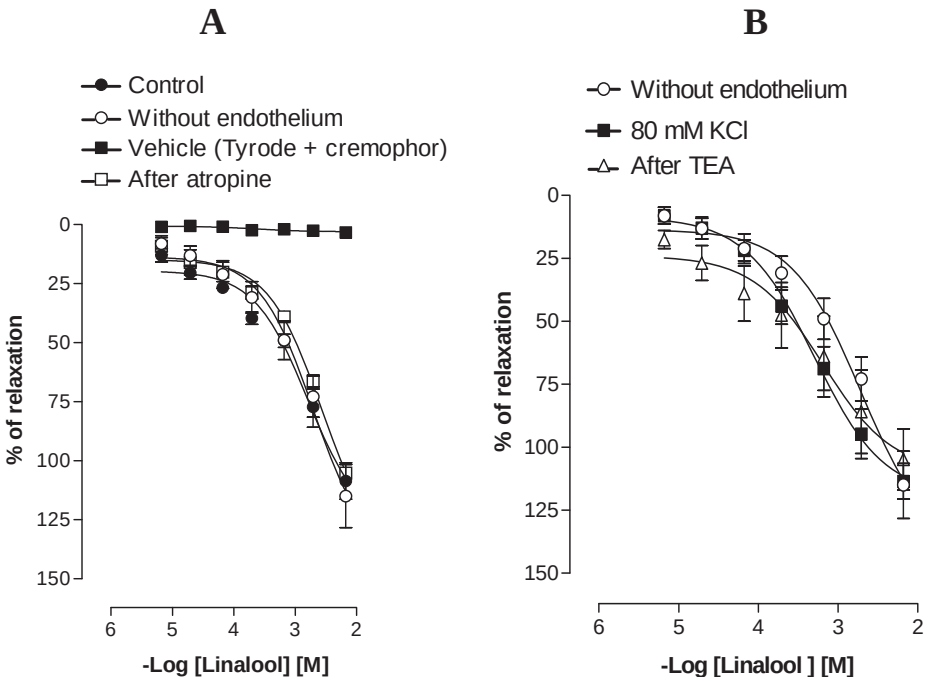


Fig. 5. Concentration-response curves for linalool in rings of rat mesenteric artery: (A) with endothelium (control), or after administration of atropine (0.01 $\mu$M), or without endothelium precontracted with Phe (10 $\mu$M), or vehicle; (B) without endothelium precontracted with Phe, or precontracted with 80 mM KCl, or without endothelium precontracted with Phe after TEA (1 mM) administration. Values are expressed as means ± S.E.M. of 6 experiments. The data were analysed by one-way ANOVA followed by Bonferroni post-test.

In a set of *in vitro* experiments with rings from the rat superior mesenteric artery, we examined whether the hypotensive response could be due to a decrease in the peripheral vascular resistance caused by a possible vasorelaxation. In the intact rings, linalool induced vasorelaxation in a concentration-dependent manner of Phe-induced tonus. This effect was not altered by atropine, a non-selective muscarinic receptor antagonist (Mitchelson, 1984), which suggests the non-participation of these receptors. In endothelium-denuded rings or after incubation with TEA, a non-selective $K^+$ channel blocker (Cook, 1989), the relaxant effect induced by linalool was not modified, suggesting that the presence of endothelium and $K^+$ channels, respectively, are not essential for the relaxant response. Thus, an endothelium- and $K^+$ channel-independent pathway is probably implicated in this effect.

Calcium is a primary regulator of tension in the vascular smooth muscle (Gurney, 1994). The maintenance of smooth muscle contraction depends on $Ca^{2+}$ influx from extracellular space through voltage- and/or receptor-operated calcium channels ($Ca_v$ and/or ROCCs, respectively) (Münzel *et al.*, 2003).

It is also known that the increase in external $K^+$ concentration (80 mM KCl) induces smooth muscle contraction through $Ca_v$ activation. This contraction is inhibited by $Ca^{2+}$ channel blockers or by removal of external $Ca^{2+}$ and is, therefore, entirely dependent on the $Ca^{2+}$ influx (Karaki and Weiss, 1998). Linalool was able to induce vasorelaxations in endothelium-denuded rings precontracted with $K^+$-depolarizing solutions (80 mM KCl) similar to those observed in rings precontracted with Phe. This result suggests that linalool could inhibit $Ca^{2+}$ influx through $Ca_v$.

Furthermore, linalool antagonized the contractions induced by $CaCl_2$. As reported by Chan *et al.* (2000), NIF also inhibits the concentration-response curve for $CaCl_2$, which strongly supports the notion that linalool could be acting as a calcium channel blocker.

Although linalool appears to be acting through the decrease of $Ca^{2+}$ influx through the plasma

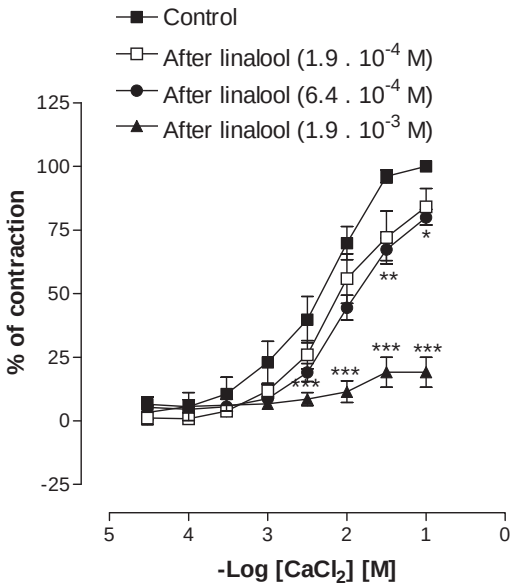


Fig. 6. Concentration-response curves for $CaCl_2$ before (control) and after incubation with linalool in rings of rat mesenteric artery without endothelium. Values are expressed as means ± S.E.M. of 6 experiments. The data were analysed with repeated measures two-way ANOVA followed by Bonferroni post-test. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs. control.

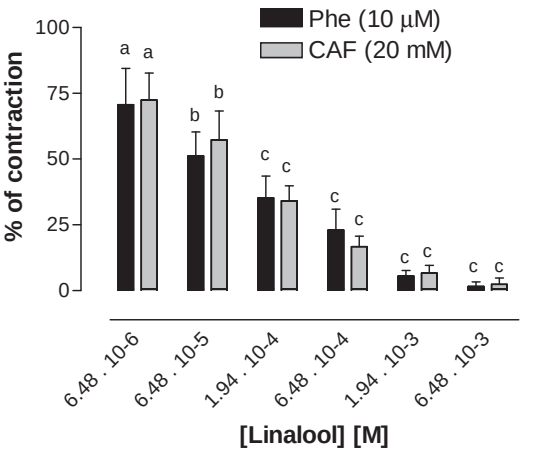


Fig. 7. Effects of linalool on transient contractions induced by 10 mM Phe or 20 mM CAF in $Ca^{2+}$-free Tyrode's solution in isolated rat mesenteric rings without endothelium. Values are means ± S.E.M. of 6 experiments. To evaluate statistic differences between dose effects and basal contraction, one-way ANOVA was used followed by Bonferroni post-test: $^{a}p < 0.05$, $^{b}p < 0.01$, or $^{c}p < 0.001$ vs. basal contraction. To evaluate statistic differences between groups, repeated measures two-way ANOVA was used followed by the Bonferroni post-test.

membrane, the inhibition of calcium release from intracellular stores could also be involved in linalool-induced relaxation. The activation of phosphoinositide turnover in response to receptor activation, as is the case with alpha-adrenoceptor activation by Phe, is crucial to the increase in cytoplasmic calcium concentration through calcium release from intracellular stores and subsequent contraction. Phe induces a rapid and transient increase in inositol triphosphate ($IP_3$) concentration in vascular smooth muscles, which causes release of calcium from $IP_3$-sensitive calcium intracellular stores (Somlyo *et al.*, 1985; Missiaen *et al.*, 1994). Furthermore, CAF could activate the ryanodine receptor which would also lead to intracellular $Ca^{2+}$ release (Missiaen *et al.*, 1994).

Linalool inhibited the transient contractions induced by Phe and CAF, suggesting that linalool also interferes with the calcium mobilization from both $IP_3$- and CAF-sensitive calcium intracellular stores.

Whereas linalool appears to be acting through the decrease of $Ca^{2+}$ influx, and calcium release from both $IP_3$- and CAF-sensitive calcium intracellular stores, other pathways cannot be discarded, like, for example, interactions between $Ca^{2+}$ and the involvement of contractile proteins in the cytoplasm of vascular smooth muscle cells, which needs to be examined in further experiments.

It is known that other monoterpenes produce effects similar to those of linalool. Bastos *et al.* (2009) showed that citronellol reduces the blood pressure by a direct effect on the vascular smooth muscles leading to vasodilation. Lahlou *et al.* (2002b) reported that terpinen-4-ol, the main monoterpene constituent of *Alpinia zerumbet* essential oil, promotes hypotension that can be attributed to a direct vasorelaxant action. Aydin *et al.* (2007) found that carvacrol, another monoterpene constituent of many essential oils, has hypotensive and vasorelaxant actions, possibly by blocking vascular L-type calcium channels. Lahlou *et al.* (2001) and Guedes *et al.* (2004) demonstrated that rotundifolone, a monoterpene from *Mentha* x *villosa* essential oil, has hypotensive activity by decreasing the peripheral vascular resistance caused by vasorelaxation. Lahlou *et*

*al*. (2002a) related this effect to the inhibition of voltage-dependent $Ca^{2+}$ channels and intracellular $Ca^{2+}$ release.

In summary, our results demonstrate that linalool has antihypertensive activity. The decrease in blood pressure is likely caused by a direct effect on the vascular smooth muscles resulting in vasodilation.

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