Biologia Geral e Experimetal

Universidade Federal de Sergipe

São Cristóvão, SE 5 (1): 12-18 30.x.2004

CARDIOVASCULAR EFFECTS INDUCED IN RATS BY THE ESSENTIAL OIL OF *OCOTEA DUCKEI* VATTIMO (LAURACEAE)

Renildo Moura da Cunha[1]
Silvia Renata Queiroz de Farias[1]
José Crispim Duarte[1]
Márcio Roberto Viana dos Santos[1]
Êurica Adélia Nogueira Ribeiro[1]
Isac Almeida de Medeiros[1]

ABSTRACT

The cardiovascular effects induced in rats by the essential oil of the leaves of *Ocotea duckei* Vattimo (ODEO) were evaluated in this study. ODEO induced hypotension, apparently due to a decrease of the peripheral vascular resistance, and bradycardia, likely due to indirect activation of the cardiac muscarinic receptors via vagal stimulation. Furthermore, ODEO induced negative chronotropic and inotropic effects on rat atria, probably due to inhibition of calcium influx, which may also contribute to the ODEO-induced bradycardia.

Key words: *Ocotea duckei*, essential oil, hypotension, bradycardia, isolated rat atria.

RESUMO

Os efeitos cardiovasculares induzidos pelo óleo essencial das folhas de *Ocotea duckei* Vattimo (OEOD) em ratos foram avaliados. OEOD induziu hipotensão, devido à diminuição da resistência periférica vascular, e bradicardia, devido à ativação indireta dos receptores muscarínicos cardíacos via estimulação vagal. OEOD também induziu efeitos cronotrópico e inotrópico negativos em átrio de rato, devido à inibição do influxo de cálcio, a qual parece contribuir também para a bradicardia induzida pelo OEOD.

Palavras-chave: *Ocotea duckei*, óleo essencial, hipertensão, bradicardia, átrio isolado de rato.

INTRODUCTION

The use of medicinal plants for the treatment of human diseases has increased considerably worldwide. Evaluation of the effects of these plants on organs and systems contribute to the development of the scientific basis for their therapeutic application, and also enrich considerably the therapeutic arsenal for the treatment of a number of diseases (Elizabetsky, 1986).

Ocotea duckei Vattimo (Lauraceae) is a medicinal plant known popularly in Brasil as "louro-de-cheiro", "louro-pimenta" or "louro-canela". Several species of the *Ocotea* genus are used in the folk-medicine of northern and northeastern Brasil for the treatment of disorders such as neuralgia, dyspepsia, anorexia and pain.

A preliminary phytochemical screening of the species revealed the presence of benzylisoquinoline alkaloids and furofuranic lignanes. This plant's essential oil (ODEO) presents 28 components, among them á-pinene, which has smooth muscle relaxant

[1] Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba, caixa postal 5009, João Pessoa, PB, 58.051-970, isacmed@uol.com.br

activity (El Tantawy *et al.*, 1999) and trans–caryophyllene – the major compound – which presents calcium channel blocker activity in isolated cardiac ventricular cells (Sensch *et al.*, 1993). Considering that no pharmacological studies of the activity of this essential oil on the cardiovascular system are available, this study aimed to evaluate the cardiovascular effects induced by ODEO in rats.

MATERIAL AND METHODS

Preparation of the essential oil from the leaves of *O. duckei*: The leaves were collected in January 2000, near the town of Santa Rita, in the Brasilian State of Paraíba. The voucher specimen is deposited in the Herbarium Prof. Lauro Pires Xavier under identification code Agra 4309. Essential oil was obtained from fresh leaves by steam distillation in a clevenger apparatus for 3 h (Matos *et al*., 1999) and stored at 4°C. When required, the oil was dissolved in a saline/cremophor (0.2% v/v) solution and diluted in saline to the desired concentrations.

Animals: Male Wistar rats (250-350 g) were used for all experiments. The animals were housed under conditions of controlled temperature (21 ± 1ºC) and lighting (lights on: 06:00-18:00 h), with free access to food (Purina-Brasil) and tap water.

Drugs: The drugs used were: heparin sodium salt (Roche), sodium thiopental (Cristália) and atropine sulfate, N^{w}-nitro-L-arginine methyl ester (L-NAME) and sodium nitroprusside (all from Sigma). All drugs were dissolved freely in distilled water.

Direct blood pressure measurements in non-anaesthetized rats: Under sodium thiopental anaesthesia (45 mg/kg, i.v.), the lower abdominal aorta and inferior vena cava were canulated via the left femoral artery and vein using polyethylene catheters. Ther catheters were then filled with heparinized saline solution and led under the skin to emerge between the scapulae. Arterial pressure was measured after 24 h by connecting the arterial catheter to a pre-calibrated pressure transducer (Statham P23 ID; Gould, Cleveland, OH, USA) coupled to an amplifier-recorder (Model TBM-4M, WPI, Sarasota, FL, USA.) and connected to a computer equipped with an analog-digital converter board (CIO-DAS16/JR, Computer Boards, Inc., Mansfield, MA, USA) and CVMS software (WPI, Sarasota, FL, USA). The data were sampled at a frequency of 500 Hz. For each cardiac cycle, the computer calculated mean arterial pressure (MAP) and heart rate (HR) (pulse interval). The venous catheter was inserted for drug administration. Sodium nitroprusside (10 µg/kg) was injected to check the efficacy of venous catheter insertion.

After cardiovascular parameters had stabilized, MAP and HR were recorded before (baseline values) and after i.v. administration of randomized doses of ODEO (1, 5, 10 and 15 mg/kg). Dose-response curves were then obtained. Successive injections were separated by a time interval sufficient to allow full recovery of cardiovascular parameters. Similar records were obtained after separate acute administration of atropine (2 mg/kg; i.v.; 15 min.), a non-selective antagonist of muscarinic receptors (Mitchelson, 1984) or L-NAME (20 mg/kg, i.v. 30 min.), a competitive inhibitor of NO-synthase (Moncada & Higgs, 1993).

Direct blood pressure measurements in anaesthetized and vagotomized rats: The animals were canulated as described above, maintained under anaesthesia with sodium thiopental (45 mg/g; i.v.) and controlled body temperature using an electric blanket (35 ± 1°C). An intra-tracheal probe coupled to an artificial ventilator (Rodent Ventilator, Ugo Basile) was inserted in these animals, which were separated into two groups. The first group was sham-operated (SHAM), while a cervical bilateral vagotomy was performed on the members of the second group.

Once cardiovascular parameters had stabilized following the surgical procedure, MAP and HR were

recorded before (baseline values) and after administration of randomized doses of ODEO (1, 5, 10 and 15 mg/kg). Dose-response curves were constructed as described above.

Rat atrium preparation: The animals were killed by stunning and bled. The left and right atria were cut perpendicular to the axis of the heart and suspended by cotton threads in organ baths containing 10 ml of Krebs´s bicarbonate solution (composition in mM: NaCl 118.0, KCl 4.7, $CaCl_2.2H_2O$ 2.5, $NaHCO_3$ 25.0, glucose 10.0, $MgCl_2.7H_2O$ 1.2 and $NaH_2PO_4H_2O$ 8.5; pH 7.4) maintained at 37°C and gassed with a carbogenic mixture (95% O_2 and 5% CO_2). Next, each atrium was stabilized under a resting tension of 0.5 g for at least 1 h. The left atrium was driven electrically by two parallel platinum electrodes with rectangular pulses at a frequency of 3 Hz, duration of 3 ms and voltage of 1.5 times the excitation threshold for each atrium. Isometric tension was recorded using a force transducer (Ugo basile, Comerio, VA, Italy) coupled to a physiograph (Gemini 2, Ugo Basile, Comerio, VA, Italy). After stabilization, ODEO (0.1, 1, 10, 30, 100, 300 and 500 μg/mL) was administrated cumulatively in the bath to obtain a concentration-response curve before (control) and after 1 μM of atropine. Concentration-response curves were also obtained for $CaCl_2$ (0.8, 1.6, 3.2, 6.4, 9.6 and 12.8 mM) before (control) and after incubation of the preparations with individual concentrations of ODEO (1, 10, 30 and 100 μg/mL).

Statistical analysis: Values are expressed as means ± SEM. When appropriate, Student´s t tests and two-way analyses of variance (ANOVA) were conducted in order to evaluate the significance of the differences between means. All statistical analyses were done using GraphPad Prism™ 3.0, GraphPad Software Inc., San Diego, CA.

RESULTS

Table 1 shows baseline values of MAP and HR in normotensive non-anaesthetized rats before (control) and after acute administration of atropine (2 mg/Kg; i.v.) or L-NAME (20 mg/kg; i.v.). The acute administration of atropine significantly increased the HR, while acute administration of L-NAME increased the MAP but reduces HR.

Table 1. Baseline values of Mean Arterial Pressure (MAP) and Heart Rate (HR) in non-anaesthetized rats before (control) and after acute administration of atropine or L-NAME.

	Control	After atropine	After L-NAME
MAP (mmHg)	117 ± 1	121 ± 4	142 ± 1 *
HR (bpm)	354 ± 4	422 ± 10 *	305 ± 7 *

Values are mean ± SEM of 6 experiments.
* $p<0.05$ vs control.

ODEO (1, 5, 10 and 15 mg/kg i.v randomly) induced hypotension (7±1, 15±2, 21±1 and 37±3%, n=6, respectively) and bradycardia (2±0.3, 9±1, 18±4 and 53±4%, n=6, respectively) in a dose-dependent manner (Figure 1). The hypotensive response was not affected after atropine (2 mg/kg, i.v.) or L-NAME (20 mg/kg, i.v.) whereas the bradycardic response was eliminated after atropine, but was not affected after L-NAME (Figure 1).

Table 2 shows baseline values of MAP and HR in control normotensive anaesthetized (SHAM) and vagotomized rats. In these animals, only the HR value increased significantly after vagotomy. Furthermore, anaesthesia with sodium thiopental did not affect significantly the ODEO-induced hypotension and bradycardia when compared with that induced in non-anaesthetized rats (10±1, 29±4, 31±5, 50±8% and 2±0.3, 16±4, 20±3, 43±6%, respectively, n=6).

Bilateral cervical vagotomy did not affect the

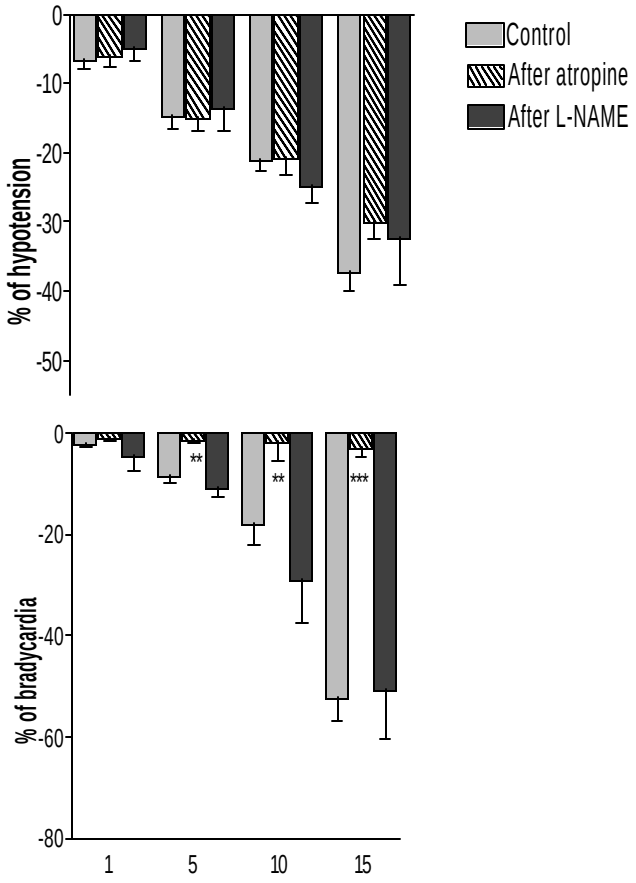

Figure 1. Hypotensive and bradycardic response induced by OEOD in non-anaesthetized rats before (control) and after acute administration of atropine (2 mg/Kg, i.v.) or L-NAME (20 mg/Kg, i.v). Values are mean ± SEM of six experiments. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ vs control.

Table 2. Baseline values of Mean Arterial Pressure (MAP) and Heart Rate (HR) in anaesthetized rats before (SHAM) and after bilateral cervical vagotomy (vagotomized).

	SHAM	Vagotomized
MAP (mmHg)	121± 3	122 ± 5
HR (bpm)	403 ± 10	472 ± 12 *

Values are mean ± SEM of 6 experiments.
* $p<0.05$ vs SHAM.

hypotensive response (11±2, 34±2, 46±5 and 51±0.7 %, respectively, n=6), whereas the bradycardic response was strongly reduced (1±0.2, 4±2, 6±1 and 10±2 %, respectively, n=6; Figure 2).

In rat atria, ODEO (0.1, 1, 10, 30, 100, 300 and 500 mg/mL) induced negative chronotropic and inotropic effects that were not affected after 1 mM atropine (figure 3). In addition, $CaCl_2$ induced positive chronotropic and inotropic effects that were attenuated after pre-incubation with ODEO (1, 10, 30 or 100 mg/mL; Figure 4a, b).

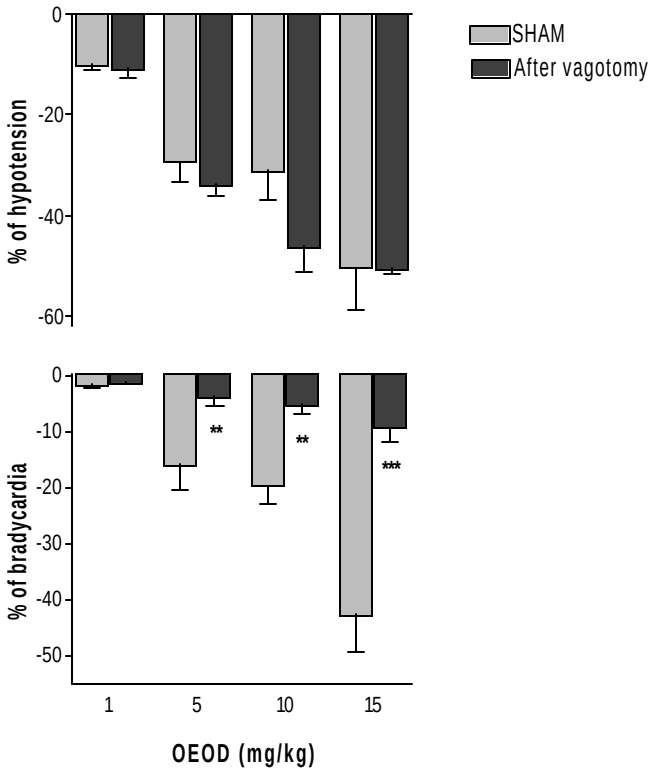

Figure 2. Hypotensive and bradycardic response induced by OEOD in anesthetized rats before (SHAM) and after bilateral cervical vagotomy. Values are mean ± SEM of 6 experiments. ** $p<0.01$ and *** $p<0.001$ vs SHAM.

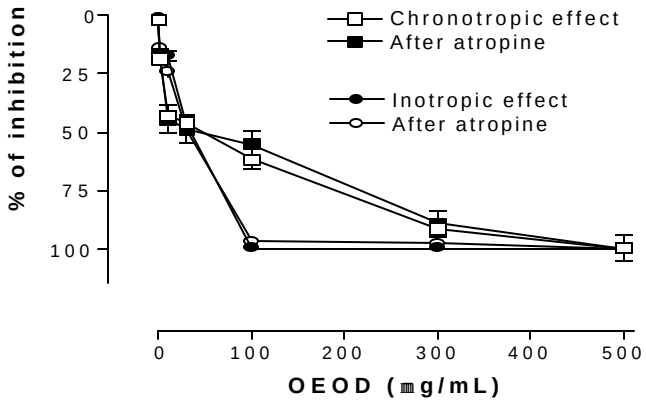

Figure 3. Chronotropic and inotropic negative effects induced by OEOD (1, 10, 30, 100, 300 and 500 µg/mL) in isolated rat atria before and after atropine. Values are mean ± SEM of six experiments.

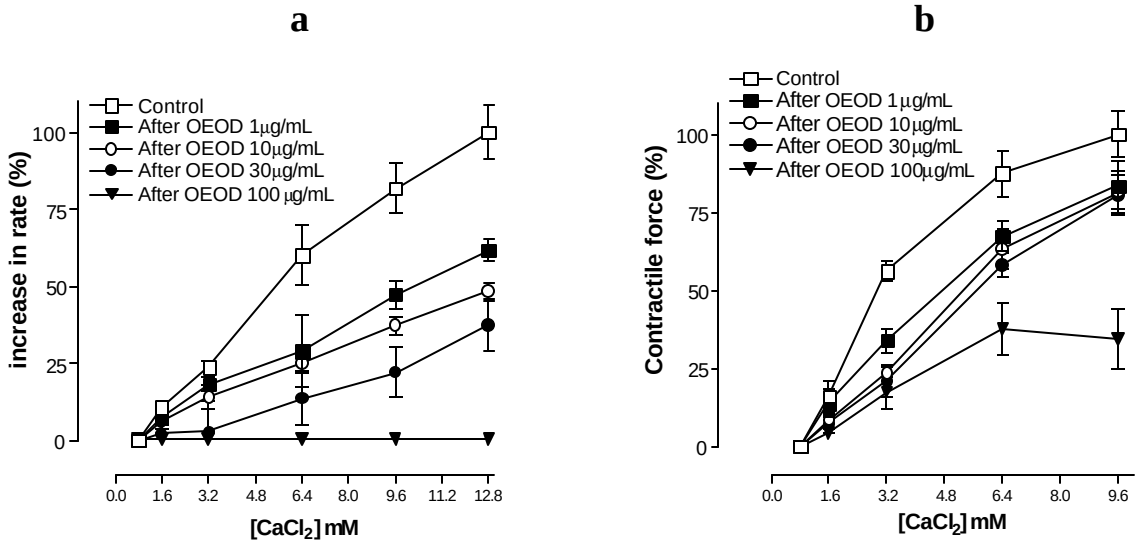

Figure 4. Effect of the incubation with OEOD (1, 10, 30 and 100 µg/mL) on chronotropic (a) and inotropic (b) positive effects induced by $CaCl_2$ (0.8, 1.6, 3.2, 6.4 and 9.6 mM) in isolated rat atria. Values are mean ± SEM of six experiments.

DISCUSSION

The major finding of the present study is that ODEO induced hypotension and bradycardia in normotensive rats, apparently due to a decrease in peripheral vascular resistance and indirect activation of cardiac muscarinic receptors, respectively. We decided to evaluate the ODEO effects first on cardiovascular parameters in non-anaesthetized rats in order to avoid the influence of anaesthesia and stress (Smith & Hutchins, 1980; Fluckiger *et al*., 1985). In these animals, ODEO induced hypotension associated with intense bradycardia.

It is well established that the primary autonomic regulation of sinoatrial node function is by vagal action via stimulation of cardiac muscarinic receptors (Peterson *et al*., 1984). Stimulation of these receptors induces intense bradycardia followed by hypotension due to a decrease in cardiac output. These receptors are predominantly of the M_2 subtype (Caulfield, 1993;

Brodde & Michel, 1999), as confirmed by the localization of M_2 mRNA in the rat heart by *in situ* hybridization (Hoover *et al.*, 1994). Although the expression of M_1, M_3 and M_4 subtype muscarinic receptor genes in the mammalian heart has been reported, they have not yet been associated with any functional response in rat atria (Stengel *et al*., 2000).

In order to evaluate the role of these receptors in ODEO-induced responses, we performed experiments in the presence of atropine, a non-selective antagonist of muscarinic receptors (Mitchelson, 1984). Under these conditions, only the bradicardic response was eliminated. We may thus conclude that ODEO could induce bradycardia via two pathways: either directly in these receptors or indirectly via vagal activation and release of acetylcholine in the sinoatrial node. This was investigated by using anaesthetized cervical bilateral vagotomized rats. In these animals, the bradicardic response to ODEO was strongly attenuated, suggesting that the bradicardic response is due to an indirect than direct activation of cardiac muscarinic receptors. These results also suggest that hypotension do not appear to be due to a decrease of cardiac output as a function of the intense bradycardia, but is probably due to a decrease in peripheral vascular resistance.

It is well established that NO is a major endothelium-derived relaxing factor, both *in vivo* and *in vitro* (Moncada *et al*., 1991), and that the release of NO from endothelial cells leads to relaxation of vascular smooth muscle cells and plays a critical role in the maintenance of vascular tone (Moncada *et al.*, 1991; Moncada & Higgs, 1993). It is thus reasonable to assume that the decrease in blood pressure is due to NO release from the vascular endothelium and the subsequent reduction of peripheral vascular resistance.

In order to investigate the possible involvement of NO in the ODEO-induced hypotensive response, we performed experiments in non-anaesthetized rats pre-treated with L-NAME, an inhibitor of NO-synthase (Moncada & Higgs, 1993). In this condition, the effects of ODEO were not altered, suggesting that NO do not appear to be involved in the ODEO-induced hypotensive response.

To rule out the hypothesis that ODEO is acting directly on cardiac muscarinic receptors to produce the bradicardic response, we used isolated rat atria. In these preparations, ODEO-induced negative chronotropic and inotropic effects that were not affected by atropine, confirming that bradycardia OEOD do not really appear to be due to direct cardiac muscarinic receptors activation. Furthermore, these effects do not appear to be essential to the expression of the bradycardic response induced by OEOD in non-anaesthetized rats, but can contribute to the bradycardia.

The Ca^{2+} channels in many different cell types are activated upon membrane depolarization and mediate Ca^{2+} influx in response to action potentials. The Ca^{2+} entering cells through voltage-operated Ca^{2+} channels serves as the second messenger of electrical signaling, initiating intracellular events, such as contraction. Multiple types of Ca^{2+} channels have been identified by their physiological and pharmacological properties (Catterall, 2000). In cardiac muscle, the major expressed Ca^{2+} channels have been designated as L-type (Larsen *et al*., 2002). Their functional activity is decreased by Ca^{2+} channel antagonists (McDonald *et al*., 1994).

As trans–caryophyllene, the major compound found in ODEO could be due to a Ca^{2+} channel blocker properties in isolated cardiac ventricular cells (Sensch *et al*., 1993), it is possible to hypothesize that the negative chronotropic and inotropic effects induced by ODEO could be due to a Ca^{2+} channel blockade. To evaluate this possibility, we recorded concentrations-response curves to $CaCl_2$ in the presence of the oil. Thus, $CaCl_2$ produced positive chronotropic and inotropic effects that were significantly attenuated or eliminated by ODEO, suggesting that ODEO acts via inhibition of the Ca^{2+} influx in isolated rat atria.

In conclusion, the results demonstrate that ODEO induces hypotension, apparently due to a

reduction in peripheral vascular resistance, and bradycardia, related to indirect activation of the cardiac muscarinic receptors via vagal stimulation. ODEO-induced negative chronotropic and inotropic effects in rat atrium, probably due to inhibition of Ca^{2+} influx, which also appears to contribute to the ODEO-induced bradycardia.

Acknowledgements: This research was supported by CAPES and PRONEX/CNPq, Brasil.

REFERENCES

Brodde, O.E. & M.C. Michel, 1999. Adrenergic and muscarinic receptors in the human heart. **Pharmacol. Rev** 51:651-690.

Catterall, W.A. 2000. Structure and regulation of voltage-gated Ca^{2+} channels. **Annu. Rev. Cell. Dev. Biol.** 16:521-555.

Caulfield, M.P. 1993. Muscarinic receptors: characterization, coupling and function. **Pharmacol. Ther.** 58:323-331.

El Tantawy, M.E., F.S. El Sakhawy, M.A. El Solí & S.A. Ross, 1999. Chemical composition and biological activity of the essential oil of the fruit of *Taxodium distichum* (L.) rich growing in Egypt. **J. Essent. Oil Res.** 11(3):386-392.

Elizabetsky, E. 1986. New directions in ethnopharmacology. **J. Etnobiol.** 6:121.

Fluckiger, J.P., M. Sonnay, N. Boillat & J. Atkinson, 1985. Attenuation of the baroreceptor reflex by general anesthetic agent in the normotensive rat. **Eur. J. Pharmacol.** 109:105-109.

Hoover, D.B., R.H. Baisden & S.X. Xy-Moy, 1994. Localization of muscarinic receptor mRNAs in rat heart and intrinsic cardiac ganglia by in situ hibridization. **Circ. Res.** 75:813-820.

Larsen, J.K., J.W. Mitchell & P.M. Best, 2002. Quantitative analysis of the expression and distribution of calcium channel subunit mRNA in the atria and ventricles of the rat heart. **J. Molec. Cell. Cardiol.** 34:519-532.

Matos, F.J.A., M.I.L. Machado, A.A. Craveiro, J.M. Barbosa-Filho, E.V.L. Cunha & C.A. Hiruma, 1999. Essential oil of Mentha x villosa from Northeastern Brazil. **J. Essent. Oil Res.** 11:41-44.

McDonald, T.F., S. Pelzer, W. Trautwein & D. Pelzer, 1994. Regulation and modulation of calcium channels in cardiac, skeletal and smooth muscle cells. **Physiol. Rev.** 74(2):365–507.

Mitchelson, F. 1984. Heterogeneity in muscarinic receptors: evidence from pharmacological studies with antagonists. **Trends Pharmacol. Sci. (suppl.)** 5:12-16.

Moncada, S. & E.A. Higgs, 1993. The L-arginine-nitric oxide pathway. **New England Journal of Medicine** 29:2002-2012.

Moncada, S., R.M.J. Palmer & E.A. Higgs, 1991. Nitric oxide: physiology, pathophysiology and pharmacology. **Pharmacol. Rev.** 43:109-142.

Peterson, G.L., G.S. Herron, M. Yamaki, D.S. Fulllerton & M.I. Schimerlik, 1984. Purification of the muscarinic acetylcholine receptor from porcine atria. **Proc. Natl. Acad. Sci. USA** 81:4993–4997.

Sensch, O., W. Vierling, W. Brandt & M.Reiter, 1993. Calcium-channel blocking effect of constituents of clove oil. **Planta Med. Suppl.** 59(7):A687-A691.

Smith, T.L. & P.M. Hutchins, 1980. Anesthetic effects on hemodynamics of spontaneously hypertensive and Wistar-Kyoto rats. **Am. J. Physiol.** 238:H539-H544.

Stengel, W.P., J. Gomeza, J. Wess & L.M. Cohen, 2000. M_2 and M_4 receptor knockout mice: muscarinic receptor function in cardiac and smooth muscle in vitro. **J. Pharmacol. Exper. Ther.** 292(3):877-885.

Aceito: 2.iii.2004