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Vasorelaxant effect of labdane-type diterpene isolated from *Copaifera langsdorfii* in the isolated rat aorta.

Janaina Ap. Simplicio[1*]; Sérgio R. Ambrosio[2]; Evelyn C. Carnio[3]; Carlos Renato Tirapelli[4].

[1] Faculty of Medicine of Ribeirão Preto-USP; [2] Nucleus of Research in Sciences and Technology/ University of Franca; [3,4] Laboratories of Physiology and Pharmacology/ College of Nursing of Ribeirão Preto-USP. [*]jasimplicio@usp.br

Introduction: *Copaifera langsdorfii* is a large tree that grows well in the north and northeastern part of Brazil. Pthytochemical studies carried out on the oleo-resin from *Copaifera langsdorfii* revealed the presence of a mixture of diterpenes. Compounds diterpenoids are the main constituents of plant extracts that are used in folk medicine for the treatment of hypertension. Labdane-type diterpenes are described to exert antispasmodic and relaxant action in vascular tissues. **Objectives:** The present investigation aims to evaluate the mechanisms underlying the cardiovascular effects displayed by the labdane *ent*-3-acetoxy-labda-8(17),13-dien-15-oic acid (labda-15-oico) isolated from *Copaifera langsdorfii*. **Methods:** Male Wistar rats (200 and 250 g) were used in accordance with the Ethical Animal Committee from the Campus of Ribeirão Preto—University of São Paulo (Protocol: 09.1.1007.53.0).The thoracic aorta was isolated for vascular reactivity experiments. cAMP and cGMP were measured by enzyme immunoassay (EIA). Nitrate was measured performed by chemioluminescence. Statistical analysis was performed using one-way analysis of variance (ANOVA). P values of less than 0.05 were considered significant. **Results:** No differences were found between the two periods of incubation (30 and 60 min), indicating that labda-15-oico achieve its maximal inhibitory action at 30 min. E_{max} values for phenylephrine in endothelium-intact (E^+) rings were reduced in the presence of labda-15-oic acid at 100 µmol/L. In endothelium-denuded (E^-) rings, labda-15-oic acid reduced phenylephrine-induced contraction at 50 and 100 µmol/L. The E_{max} values for serotonin in E^+ and E^- rings were reduced in the presence of the labdane at 10, 50 and 100 µmol/L. The Labda-15-oic acid inhibited the contraction induced $CaCl_2$ in E^- rings in the concentration of 10, 50 and 100 µmol/L, after stimulation with phenylephrine (0.73±0.07g, n=6; 0.79±0.12g, n= 5; 0.41±0.06g, n=6), compared to control (1.28±0.10g, n=6), or with KCl (0.91±0.13g, n=5; 0.78±0.14g, n=7; 0.67±0.05g, n=6), compared to control (1.42±0.06g, n=8). The labdane induced relaxation in E^+ and E^- rings pre-contracted with phenylephrine or KCl . The inhibitory effect elicited by lada-15-oico was totally abolished 60 and 120 min after the removal of the labdane from the medium bath in E- and E+ rings, respectively. In E^+ rings pre-contracted with phenylephrine, the E_{max} values for relaxation were reduced in the presence of L-NAME, ODQ, haemoglobin, RP-8-Br-Pet, 4-amynopiridine, Apamine and glibenclamide. There was a rightward displacement of the concentration–response curve for labda-15-oico in the presence of L-NAME, ODQ, haemoglobin, 7-nitroindazole, Rp-8-Br-Pet, thapsigargin, tetraethylammonium, 4- amynopiridine, Apamine, Charybdotoxin and glibenclamide. On the other hand, indomethacin, wortmannin, LY294002, atropine, propranolol, H89 and SQ22536 did not have a significant effect on labdane induced relaxation. The labdane increased the levels of cGMP and nitrate but not cAMP in E^+ rings. **Conclusion**: The effect of labda-15-oico is reversible and involves different pathways. The labdane acts on vascular smooth muscle where it blocks Ca^{2+} influx through interference with both voltage and receptor-operated channels. The relaxant action of the labdane is also partly mediated by the activation of endothelial NO-cGMP pathway and opening of K^+ channels.

Keywords: Diterpenes, Cardiovascular Effects, Relaxation, rat aorta**.**

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