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The *Operculina macrocarpa* (l.) urb. (jalapa) tincture modulates human blood platelet aggregation ☆

Taiana Magalhães Pierdoná [a], Nathalia Rocha Lima [b], Raony Cássio Millet Rodrigues [b], Jonas Pires Teixeira [b], Romélia Pinheiro Gonçalves [b], Juvenia Bezerra Fontenele [b], Silvânia Maria Mendes Vasconcelos [a], Glauce Socorro de Barros Viana [a], Luzia Kalyne Almeida Moreira Leal [a,b,*]

[a] *Programa de Pós-Graduação em Farmacologia, Departamento de Fisiologia e Farmacologia, Faculdade de Medicina, Universidade Federal do Ceará, Brazil*
[b] *Centro de Estudos Farmacêuticos e Cosméticos (CEFAC), Departamento de Farmácia, Faculdade de Farmácia, Odontologia e Enfermagem, Universidade Federal do Ceará 60431-327, Brazil*

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ABSTRACT

Ethnopharmacological relevance: *Operculina macrocarpa* is an ornamental climbing plant of the North-eastern Brazil extensively used in traditional medicine as depurative of the blood and for the treatment of thrombosis. To investigate the antiplatelet and anticoagulant potential of *Operculina macrocarpa* and to determine the possible mechanisms of action.

Material and methods: The *Operculina macrocarpa* tincture (OMT) was characterized by the polyphenol content and chromatographic profile established by HPLC with detection and quantification of three phenol acids (caffeic, clorogenic and gallic acids). The human platelet aggregation was induced *in vitro* by the agonists ADP, collagen, thrombin, epinephrine or arachidonic acid, and the antiplatelet effect of OMT was evaluated in the presence or absence of aspirin (a nonselective inhibitor of cyclooxygenase), pentoxifylline (a phosphodiesterase inhibitor), ticlopidine (a $P2Y_{12}$ purinoceptor antagonist) or ODQ (a selective inhibitor of guanilate cyclase). The effect of OMT on the partial thromboplastin time, prothrombin time and bleeding time were investigated on human or rat plasma.

Results: The strongest antiplatelet effect of OMT (50–400 μg/mL) was observed on the ADP- induced aggregation with inhibitions up to 55%, while among others agonists (epinephrine, collagen, thrombin and arachidonic acid) maximal inhibitions reached by OMT (200 μg/mL) were on platelet aggregation induced by collagen (18%) or epinephrine (20%). The antiplatelet effect of OMT (400 μg/mL) was comparable to aspirin, a nonspecific inhibitor of cyclooxygenase. The ticlopidine and pentoxifylline increased 5.1 and 3.8 fold the inhibitory effect of OMT on ADP-induced platelet aggregation, respectively. On the other hand, L-arginine, ODQ and aspirin showed a slightly or no effect on antiplatelet effect of OMT. The bleeding time in rats was significantly increased by OMT, but the tincture did not interfere on the activated partial thromboplastin or prothrombin time in human plasma.

Conclusions: This study showed that the tincture of *Operculina macrocarpa* has antiplatelet effect that cannot be attributed to a single biochemical mechanism and at least part of it cannot be related to the OMT inhibition of $P2Y_{12}$ purinergic receptors.

1. Introduction

The participation of platelets in hemostasis is of fundamental importance to the normal physiology. Circulating platelets in normal conditions must be able to maintain a direct and repeated contact with the vessel wall without premature activation. On the other hand, after a vascular injury, the platelets must recognize the damaged wall, adhere and form a stable clot, which will remain in place until repair is no more needed (Brass, 2010). When there is an injury to the blood vessel wall or endothelial cell layer, platelets adhere to the site of injury by binding to von Willebrand factor (vWF) and collagen fibrils in the sub-endothelial matrix. Subsequently, platelet are stimulated to secrete various molecules such as adenosine diphosphate (ADP) and thromboxane A_2 (TXA_2), which activate neighboring platelets, and increase the expression of membrane integrins including glycoprotein IIb/IIIa (GP IIb/IIIa) receptors, which form bridges between adjacent platelets by binding to fibrinogen. These processes lead to the formation of a platelet plug at the injury site (Davi and Patrono, 2007).

Thrombosis refers to a pathological formation of a hemostatic plug within the blood vessels in the absence of bleeding. Arterial

☆Chemical compounds studied in this article, caffeic acid (PubChem CID: 689043); gallic acid (PubChem CID: 370); chlorogenic acid (PubChem CID: 1794427).

* Corresponding author. Tel.: +55 85 33668294; fax: +55 85 33668257.
E-mail address: kalyne@ufc.br (L.K.A.M. Leal).

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thrombosis is an acute complication that develops in association with atherosclerosis and causes cardiovascular diseases such as ischemic heart disease and stroke (Ruggeri, 2002; Davi and Patrono, 2007). Platelets are critical components in thrombosis and may participate in the progression of the atherosclerotic plaque (Ruggeri, 2002; Jennings, 2009). Thrombotic diseases, especially heart disease and cerebral vascular thrombosis became the leading causes of death and its incidence is increasing every year (Bao et al., 2009).

Antiplatelet agents such as aspirin and clopidogrel are widely used clinically and directly inhibit platelet aggregation (Gaglia et al., 2010). However, the continuous use of these agents is limited because they can induce resistance or adverse effects such as headache, abdominal cramps, vomiting and gastric ulceration (Angiolillo et al., 2007). For these reasons, a number of studies (Tsai et al., 2000; Ballabeni et al., 2007; Ryu et al., 2009) have been conducted to search new medicines from synthetic or natural source.

Several authors have shown the antiplatelet effect of extracts or molecules from medicinal plants and their constituents, such as *Amburana cearensis* (Leal, 1995) and piplartine, an alkamide isolated from *Piper tuberculatum* (Fontenele et al., 2009).

Operculina macrocarpa (L.) Urb (Convolvulaceae), is an ornamental climbing plant of the Northeastern Brazil popularly known as "jalapa" or "batata-de-purga". The roots of this plant are mainly used in traditional medicine as depurative of the blood (Matos, 1982). In addition, a phytomedicine entitled "Aguardente Alemã" an ethanolic solution (tincture) produced from *Operculina macrocarpa* (OMT) associated with *Convolvulus scamnonia* is extensively used in folk medicine in the Northeastern Brazil for the treatment of circulatory diseases such as thrombosis (Carvalho et al., 2003). In this sense, the objective of this present study was to investigate the antiplatelet and anticoagulant effects of *Operculina macrocarpa*.

2. Materials and methods

2.1. Drugs, chemicals and phytomedicine

Adenosine diphosphate (ADP), dimethylsulfoxide (DMSO), Tween 80, acetylsalicylic acid (ASA), 1*H*-[1,2,4] oxadiazolol [4,3-a] quinoxalin-1-one (ODQ), L-arginine, caffeic acid, chlorogenic acid and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Arachidonic acid and thrombin were purchased from Chrono-log (USA). Pentoxifylline (Trental®) and ticlopidine (Ticlid®) were obtained from Sanofi-Aventis (Brazil). Sodium nitroprusside (Nitrop®) was obtained from Hypofarma (Brazil). Heparin (Liquemine®) was obtained from Roche (Brazil). Collagen and epinephrine were purchased from Helena Laboratories (USA). Trifluoroacetic acid was obtained from VETEC (Brazil), while acetonitrile was originally provided by TEDIA (Brazil). All others reagents were of analytical grade.

2.2. Samples of human blood

Blood samples were obtained from healthy volunteers who had not taken any drugs for at least fifteen days. The study was approved by the Human Research Ethics Committee of the Federal University of Ceará, Brazil, under the number 169/10.

2.3. Animals

Male Swiss mice (25–30 g) were housed in standard environmental conditions (23 ± 1 °C, humidity 60 ± 5% and a 12 h/12 h dark/light cycle-light on at 7.00 a.m.), with food and water available *ad libitum* in accordance to the Guide for the Care and Use of Laboratory Animals, US Department of Health and Human Services, 1985. All experiments were conducted in a quiet room at a constant temperature of 25 °C. All drugs were administered orally at a volume of 10 mL/kg. The experiments were approved by the Animal Research Ethics Committee, Federal University of Ceará (registered under No. 63/10).

2.4. Operculina macrocarpa tincture

The tincture from the roots of *Operculina macrocarpa* (resin content: 1.38 ± 0.47 g% w/v) was obtained from the Ravick Chemical Products and Cosmetics Laboratory (Fortaleza, CE, Brazil). The plant was collected in the city of Parnaíba (Piauí, Brazil) located at coordinates 2° 54′49″ S and 41° 47′15″ W. The botanical material was identified by the Herbarium Prisco Bezerra, Federal University of Ceará (voucher specimen registered under the number 45489).

2.5. Chemical characterization of Operculina macrocarpa

The chromatographic analysis by high-performance liquid chromatography (HPLC) of OMT was performed according the method described by Michelin (2008). The assays were performed on Alliance —Waters 2695 (Milford, MA) chromatograph with a binary pump, auto-sampler, and photodiode-array detector (Waters-2996 PDA) at 310 nm. The separations were performed with an analytical reverse-phase column C18 (Varian®, 250 × 4.6 × 5 mm) and a guard column (Phenomenex®, 4 × 3 × 5 mm) at 40 °C in a thermostatic oven. The mobile phase was made from water/trifluoroacetic acid (mobile phase A) and acetonitrile/trifluoroacetic acid (mobile phase B) in a gradient mode for 31 min (total run time) by varying the mobile phase B from 10 to 100% at 1.0 mL/min flow rate. The solvents were previously degassed under vacuum by sonication during 5 min and filtered through Phenomenex nylon membrane (0.45 μm). The samples were dissolved in the initial mobile phase and filtered through Waters PTFE membrane (0.45 μm) before injection (20 μL). The data was processed by Empower® (Waters, USA) software.

The identification of caffeic, chlorogenic and gallic acid in *Operculina macrocarpa* tincture by HPLC experiments were based on the retention time (rt) of external standards. The contents of the three phenol acids in the tincture were calculated using calibration curves. The ranges of calibration curves were 0.01–0.015 mg/mL for chlorogenic acid and gallic acid, 0.001–0.005 mg/mL for caffeic acid. The linear relationship was obtained correlating the concentration of phenols acids to the correspondent peak area. The linear regression equations were determined and the correlation coefficients for and caffeic acid, chlorogenic acid and gallic acid were 0.996, 0.998 and 0.993, respectively. The content of phenols acids were expressed as mean of three determinations and coefficient of variation.

2.6. Measurement of total polyphenol content and phytochemical prospecting in OMT

The determination of the total polyphenol contents of OMT was measured by the Folin–Ciocalteu colorimetric method (Singleton and Rossi, 1965). An aliquot (100 μL) of the OMT was mixed to 250 μL of Folin–Ciocalteu reagent (1 N). The mixture was shaken before adding 1250 μL Na_2CO_3 (20%) and adjusting with Milli-Q water to a final volume of 10 mL. After 40 min of standing in dark, the mixture was analyzed by spectrophotometry (Beckman Coulter DU 640, Germany) at 715 nm. The amount of total polyphenols was calculated through calibration curve (linear regression equation: $y = 0.077x + 0.0468$; $r = 0.9985$) of gallic acid (4–16 μg/mL). Results were expressed as gram gallic acid equivalents (GAE) per mL of OMT.

The phytochemical profile of the OMT was performed according to the method described by Matos (2009) and identification