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RESEARCH ARTICLE

Vasoactive properties of *Ceiba pentandra* in porcine coronary artery and different conductance and resistance vessels from rats: A role of nitric oxide

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ABSTRACT

Background: *Ceiba pentandra* (L.) Gaertner (Malvaceae) a plant widely spread in the world, particularly in sub-Saharan Africa where it is used to handle several pathologies like high blood pressure without scientific evidence by experimental scientific works. **Aim and Objective:** The purpose of this study is to show how a hydroethanolic leaves extract of *Ceiba pentandra* (FCP) produces relaxant effects in porcine coronary, rat mesenteric artery, carotid, femoral arteries, and thoracic aorta and to determine the mechanisms which lie beneath this effect. **Materials and Methods:** Porcine coronary, rat mesenteric, carotid, and femoral arteries and thoracic aorta rings were suspended in organ chambers for recording of changes in isometric forces. Rings with endothelium were incubated or not with L-nitroarginine to block nitric oxide (NO) synthase, manganese (III) tetrakis(1-methyl-4-pyridyl)porphyrin, polyethylene glycol catalase (CAT), an inhibitors of intracellular production reactive oxygen species (ROS); CAT, an inhibitor of extracellular ROS; wortmannin, an inhibitor of redoxsensitive pathway phosphoinositide 3-kinase (PI3 kinase)/Akt; (4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo [3,4-d] pyrimidine), an inhibitor of Src kinase; apamin, an inhibitor of small conductance potassium channels calcium dependent (SKCa) and tram-34, an inhibitor of intermediary conductance potassium channels calcium dependent (IKCa); and indomethacin, an inhibitor of cyclooxygenase before contraction with U46619 or phenylephrine and a concentrationrelaxation curve to FCP. **Results:** Some experiments show that endothelium was taken away before contraction with U46619 and concentration relaxation to FCP. The hydroethanolic leaves extract of *C. pentandra* produces a vasodilatory result in porcine coronary artery pre-contracted with U46619. This effect is endothelium dependent and is mediated by NO. FCP generates, as well, vasorelaxant results in superior mesenteric arteries, carotid arteries, thoracic aorta, and femoral arteries from rat. *C. pentandra* generates vascular relaxation which can be the explanation of the benefic effect of this plant in the treatment of high blood pressure (HBP) in Africa. **Conclusion:** *C. pentandra* holds vasorelaxants properties on pig coronary artery, thoracic aorta, the main superior mesenteric artery, and femoral and carotid artery from rat justifying its utilization in the treatment of the arterial HBP. This effect necessitates the presence of a functional endothelium and passes by the redox-sensitive pathway Src kinase and PI3-kinase/Akt.

KEY WORDS: *Ceiba pentandra*; Vasorelaxant Effects; Porcine Coronary; Rat Arteries

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INTRODUCTION

Way of life, consumption of hypercaloric or too much-salted food, tobacco or alcohol, and stress is responsible for the emergence of diseases such as obesity, diabetes, and arterial high blood pressure (HBP).[1,2] HBP represents 13.3 at 50% of cardiovascular diseases in Africa.[3,4] These epidemiological

data show that this pathology became a real problem of public health.[5,6] In Africa, most of the people use medicinal plants for their health needs.

It is the purpose of our study that concerns *Ceiba pentandra* (L.) Gaertner (*Malvaceae*) a plant widely spread in the world, particularly in sub-Saharan Africa where it is used to handle several pathologies like HBP[7-11] without scientific evidence by experimental scientific works. Thus, our study aim at evaluating antihypertensive consequences of *C. pentandra* through vasoactive properties on pig coronary arteries ring and different conductance and resistance vessels from rats and the mechanisms involved.

MATERIALS AND METHODS

Plant Material

Leaves of *C. pentandra* (L.) Gaertner (*Malvaceae*) were gathered in February 2016, in the botanical garden of the Faculty of Medicine, Pharmacy and Odontology of Cheikh Anta Diop University of Dakar (Senegal). Leaves were identified in the botanic laboratory of Fundamental Institute of Black Africa (IFAN). Voucher specimens were deposited at the herbarium's university under No. IFAN61996. The drug was dried 15 days shielded from the light before being transformed into powder and kept at ambient temperature (25–30°C), in room aerated until its routing in the Laboratory of Biophotonic and Pharmacology, Faculty of Pharmacy of University of Strasbourg (France), where the vascular reactivity experiments was achieved.

Ten grams of *C. pentandra* leaves powder was macerated in 100 ml of an aqueous-ethanolic solution (60 ml of ethanol and 40 ml of water) in an Erlenmeyer flask and mixed for 24 h at room temperature. Macerate was filtered by gravity on a funnel with cotton wool. The filtrate was collected and evaporated with a rotary evaporator under the following conditions: Temperature of the water bath 40°C was obtained by a vacuum pump Vacuubrand membrane. The crude leaves extract obtained after evaporation was directly frozen in liquid nitrogen before lyophilization. This step enabled us to obtain a solid content to achieve the vascular reactivity tests and western blot analysis.

Phytochemical Screening

Phytochemical tests were conducted on hydroethanolic crude leaves extract of *C. pentandra* to determine any presence of flavonoids, tannins, sterols, and terpenoids with standard protocols.[12,13]

Determination of Total Phenolic Contents

The total phenolic components were ascertained in triplicate and expressed as mg gallic acid equivalents (GAE) with the Folin–Ciocalteu method.[14]

Chemical Material

Nω-nitro-L-arginine (L-nitroarginine [L-NA]), indomethacin (INDO), apamin (APA), tram-34, catalase (CAT), and polyethylene glycol CAT (polyethylene glycol [PEG]-CAT) were produced from Sigma Chemical Co. (Saint Louis, MB, U.S.A). Wortmannin and the superoxide dismutase (SOD) mimetic manganese (III) tetrakis(1-methyl-4-pyridyl)porphyrin (MnTMPyP) were from Alexis chemicals. U46619 (9, 11-dideoxy-9α-methanoepoxy prostaglandin $F_{2\alpha}$) and 4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo [3,4-d] pyrimidine (PP2) from Calbiochem. Phenylephrine (PE), acetylcholine (Ach), and bradykinin were obtained from Cayman Chemical (Ann Arbor, MI, U.S.A).

Vascular Reactivity Studies

This study complies to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (publication no. 85–23, revised 1996). It was conducted from February 2016 to June 2017 at biophotonics and pharmacology laboratory of Faculty of Pharmacy of University of Strasbourg. The thoracic aorta, the main superior mesenteric artery, the carotid and femoral arteries from rats anesthetized with phenobarbital (50 mg/kg, intraperitoneally), and left circumflex coronary artery were removed and carefully cleaned of fat and connective tissue members in a physiological Krebs bicarbonate solution at 4°C. The artery was cut into rings of 3–4 mm length. In some tests, endothelium was mechanically taken away by rubbing intimal surface of rings with a notched clamp. The rings were suspended between two metal hooks in tanks separated organ of 10 ml thermostated at 37°C and oxygenated with carbogen (95% O_2 and 5% CO_2), and containing Krebs solution (composition in mM: NaCl 119, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.18, $CaCl_2$ 1.25, $NaHCO_3$ 25, and D-glucose 11, pH 7.4). Each ring was linked to an isometric tension sensor which measures the variations. To measure changes of isometric tension, each ring was maintained under a basal tension of 5 g. After an equilibration period of 60 min, rings were contracted with a Krebs solution containing 80 mM KCl to check out artery integrity. After washout and a further 30 min period equilibration, rings of rat mesenteric, carotid, femoral arteries, and thoracic aorta were contracted again with PE (1 μm) and the relaxation to Ach (1 μm) was fixed to test the integrity of the endothelium. In the same way, rings of porcine coronary arteries were contracted with thromboxane mimetic U46619 (1–60 nm) to about 80% of the maximal contraction before the addition of bradykinin (0.3 μm) to verify if there is the presence of a functional endothelium. After an equilibration period of 60 minutes, rings were contracted with a Krebs solution which holds 80 mM KCl to check out artery integrity. After washes with Krebs and at the basal tension of 5 g, rings were contracted with U46619 (10^{-8}–10^{-7} M), an analog of thromboxane A2, approximately 80% of maximal contraction before the addition of bradykinin (3.10^{-7} m) for detecting the presence of a functional endothelium. The vessels were considered to

have a functional endothelium when bradykinin produced more than 90% relaxation. Vessels were then washed again 3 times with Krebs and incubated with or not separately with various inhibitors of endothelial factors vasorelaxant (L-NA, wortmannin, PP2, INDO, APA, tram-34, MnTMPyP, CAT, and PEG-CAT) for 30 min before contracting with U46619. In this way, their sustained contractions were produced, and the relaxant effect was assessed by adding in a cumulative fashion to achieve a relaxation-concentration curve to *C. pentandra* leaves extract (FCP).

In further experiments, cumulative contractile responses induced by U46619 (1–100 nm), 5HT (10 nm–10 μm), and KCl (10–80 mm) were procured in the presence of FCP (1, 3, and 10 μg/ml) in the presence of a functional endothelium. Finally, FCP was incubated for two former conditions in the presence of L-NA (300 μm), an nitric oxide (NO) synthase inhibitor.

Statistical Analysis

Results are revealed as means ± standard error of the mean of 6–8 tests. Statistical meaning was induced through a one-way analysis of variance followed by Bonferroni's test or with Student's *t*-test for paired data as required. Statistical analysis was carried out using GraphPad Prism version 6.01® for Windows (GraphPad Software, San Diego, Calif., USA). $P < 0.05$ was considered as statistically significant.

RESULTS

Phytochemical Analysis of Crude Hydroethanolic Extract of *C. pentandra* Leaves

Reagent-based phytochemical screening of *C. pentandra* hydroethanolic leaves crude extract revealed the presence of flavonoids, tannins, sterols, and triterpenes [Table 1]. The content in total phenolic compounds was of 588.1±2.2 mg of GAE per gram of extract [Table 2].

Table 1: Class of phytochemical constituents of crude hydroethanolic extract of *C. pentandra* leaves (FCP)

Compounds screened for	FCP
Tannin	++
Flavonoid	++
Sterol and triterpene	+++

The test was positive (+) when compound screened for was detected, negative (−) when it was not found

Table 2: Concentration of total phenols in *Ceiba pentandra* extracts by Folin–Ciocalteu assay

FCP	Total polyphenolic contents (mgGAE/g)
	588.1±2.2 mg

Results are expressed as mg of gallic acid equivalent (GAE) per gram of extract under the form of mean±SEM (n=3)

FCP Induces Endothelium-dependent Relaxations in Coronary Artery Rings

Joining of cumulative concentrations of FCP on separated porcine coronary artery rings contracted with U46619 produced concentration-dependent relaxations in endothelium intact but not in endothelium-denuded arteries [Figure 1a]. The endothelium-dependent relaxations began at concentrations of >1 μg/ml and attained maximal value close to 30 μg/ml (E_{max} = 102.30 ± 2.99%). Relaxations to FCP were importantly affected by L-NA (300 μm), an inhibitor of eNOS (approximately 80% of inhibition of E_{max}) [Figure 1b]. INDO (10 μm), an inhibitor of cyclooxygenases (COXs) and the conjunction of tram-34 (1 μm) plus APA (100 nm), two inhibitors of endothelium-dependent hyperpolarization (EDH)-mediated responses have not altered the maximal relaxation [Figure 1c and d]. Besides, such findings show that FCP leads to endothelium-dependent relaxations involving NO component, while EDH and prostacyclin (PGI2)-sensitive component have not appeared to play a role in the vascular effects.

Role of Reactive Oxygen Species (ROS), Src Kinase, and the Phosphoinositide 3-Kinase (PI3-Kinase)/Akt Pathway FCP-induced Relaxations

Recent results reveal that polyphenolic compounds can produce NO-mediated relaxations in porcine coronary arteries by a mechanism involving endothelial production of ROS and activation of the PI3-kinase/Akt and Src kinase pathway,[15-18] leading to eNOS phosphorylation. The resulting activation of eNOS through this pathway has been proved to occur even in a Ca^{2+}-free medium.[19] Consequently, experiments were achieved using MnTMPyP (100 μm), a membrane-permanent SOD mimetic and CAT (500 U/ml) and PEG-CAT (500 U/ml), a membrane-permanent CAT mimetic, to verify whether there is a ROS signaling pathway in FCP-induced relaxations. The relaxations produced by FCP (1–30 μg/ml) were markedly decreased by native CAT, by the membrane-permanent SOD mimetic MnTMPyP and the cell-permeable PEG-CAT (approx. 98%, 95%, and 90% of inhibition of E_{max}, respectively) [Figure 2a and b].

In addition, it has been clearly proved that Src, a redox-sensitive kinase, acts as an upstream activator of the PI3-kinase/Akt pathway, leading to the polyphenol-induced eNOS activation.[17,18] As a result, experiments were performed to determine Src kinase/PI3-kinase/Akt pathway role in relaxations to TAE. Inhibition of Src kinase with PP2 (10 μm) and the PI3-kinase by wortmannin (30 nm) abolished relaxations to TAE (70% and 83% of inhibition, respectively) [Figure 2c and d].

FCP Inhibits the Contractile Responses Induced by U46619, 5HT, and KCl in Coronary Arteries

Exposure of coronary artery rings to FCP (1, 3, and 10 μg/ml) during 30 min before the addition of cumulative

concentrations of U46619 (1–100 nm), 5HT (10 nM–10 μm), and KCl (10–80 mm) significantly reduced contractions in rings with endothelium [Figure 3a-c]. The inhibitory effect of TAE on contractile responses cumulative concentrations of 5HT was prevented by L-NA (300 μm, 30 min), indicating the involvement of the activation of eNOS [Figure 3d].

FCP Induces Relaxations in Different Conductance and Resistance Arteries Ring from Rats

The addition of cumulative concentrations of FCP on isolated thoracic aorta, the main superior mesenteric arteries, and femoral and carotid arteries ring from rats

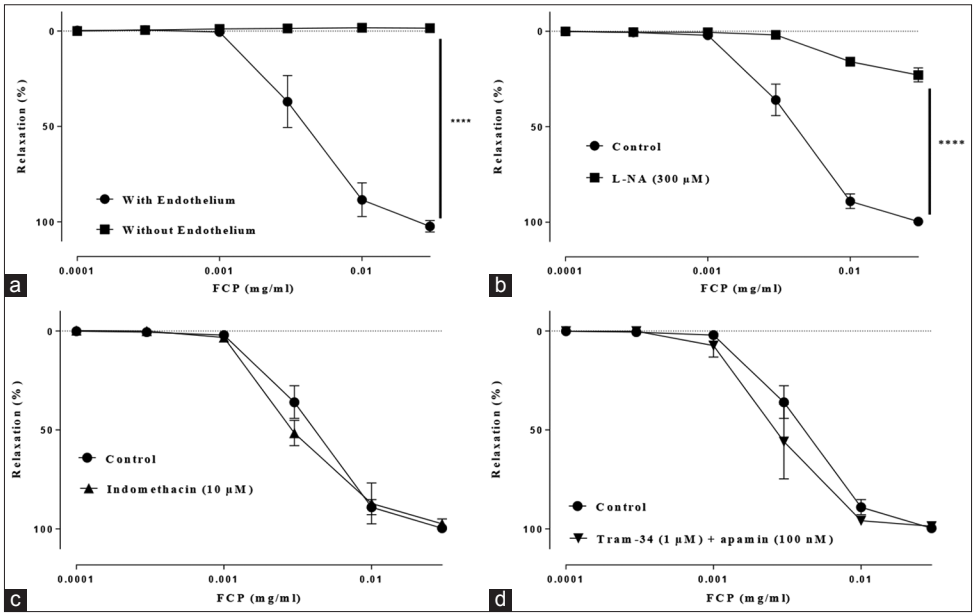

Figure 1: Effect-concentration curves of FCP in isolated porcine coronary artery. (a) Endothelium-dependent relaxations induced by FCP. (b) Effect of L-nitroarginine (300 μm). (c) Effect of indomethacin (10 μm) on FCP-induced relaxations in coronary artery rings with endothelium. (d) Relaxant effect of FCP in the presence of apamin (100 nm) plus tram-34 (1 μm) in intact arteries. Results are shown as means ± standard error of the mean of six different experiments. ****$P < 0.0001$ for inhibitory effect versus control

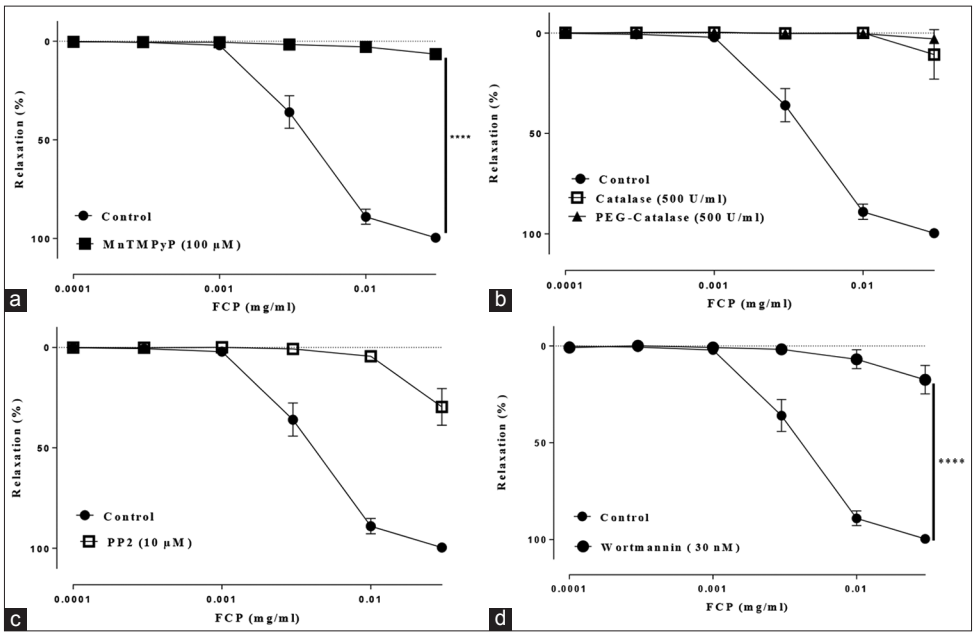

Figure 2: Role of the redox-sensitive Src kinase and the phosphoinositide 3-kinase (PI3 kinase)/Akt pathway in FCP-induced endothelium-dependent relaxations. Coronary artery rings with endothelium were incubated with MnTMPyP (100 μm), a cell permeable superoxide dismutase mimetic (a), native catalase (CAT, 500 U/ml) or polyethylene glycol (PEG-CAT, 500 U/ml), a membrane-permanent analog of CAT (b), the Src kinase inhibitor 4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo [3,4-d]pyrimidine (10 μm) (c), and the PI3-kinase inhibitor wortmannin (30 nm) (d) for 30 min before contraction to U46619 and subsequent relaxation to TAE. Results are shown as means ± standard error of the mean of six different experiments. ****$P < 0.0001$ for inhibitory effect versus control

contracted with PE-induced concentration-dependent relaxations in endothelium-intact arteries [Figure 4]. FCP effects from rats are more marked on carotid artery (71.28% at 10 µg/ml) than to main superior mesenteric artery (67.88%), thoracic aorta (49.64%), and femoral arteries ring (48.51%).

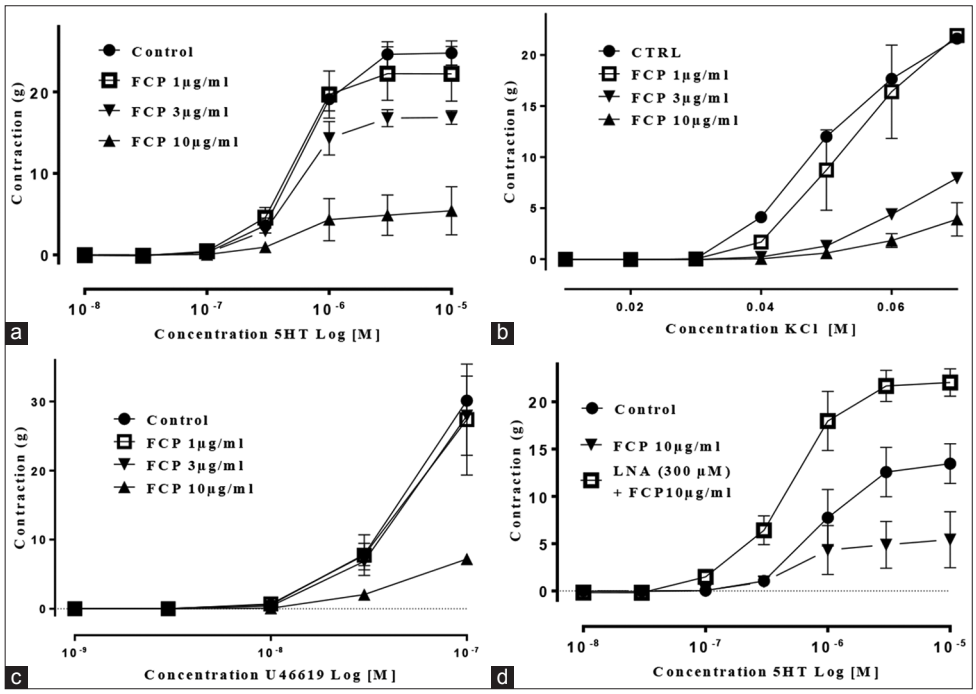

Figure 3: Effect of FCP on contractile responses in coronary artery rings with endothelium. The graph shows contractile curves to 5HT, KCl, and U46619 assessed either in the presence of FCP (1, 3, and 10 µg/ml) (a-c). The graph shows contractile curves to 5HT assessed either in the presence or absence of FCP (10 µg/ml), with or without L-nitroarginine (300 µm, 30 min; Tram-34 1 µm plus apamin 100 nm, 30 min; and indomethacin 10 µM). (d) Results are shown as means ± standard error of the mean of eight different experiments

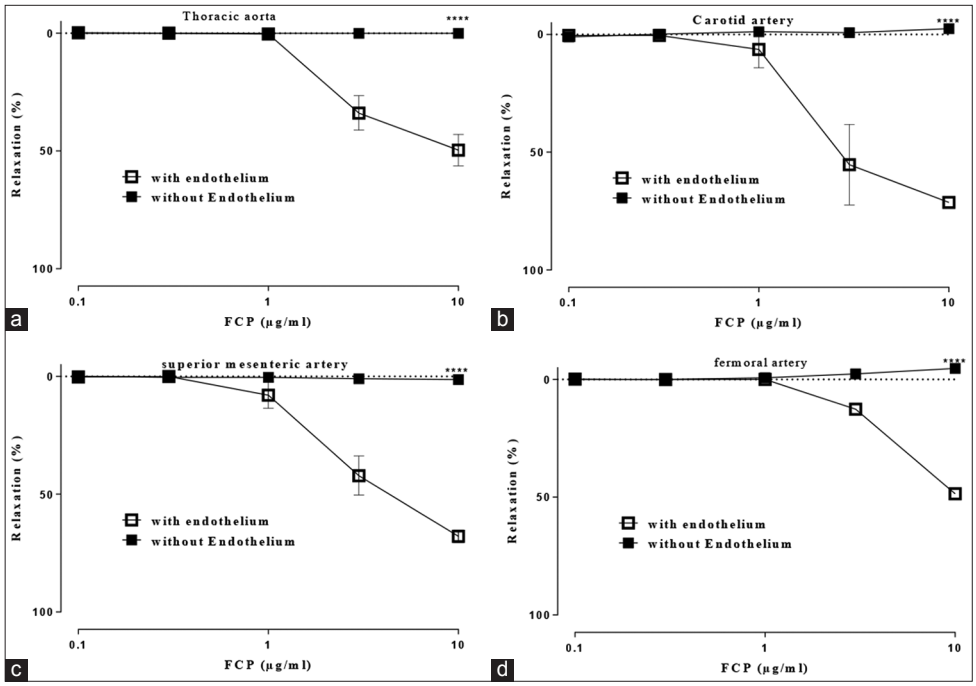

Figure 4: Characterization of FCP-induced relaxations in the thoracic aorta, the main superior mesenteric artery, and the carotid and femoral artery from rats. Rings with and without endothelium were contracted by phenylephrine before the addition of cumulative concentrations of FCP (0.1–10 µm). (a) Relaxations effects induced by FCP in intact thoracic aorta. (b) Relaxations effects induced by FCP in carotid artery with endothelium. (c) Relaxations effects induced by FCP in intact main superior mesenteric artery. (d) Relaxations effects induced by FCP in intact femoral artery from rats. Results are shown as means ± standard error of the mean of six different experiments. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$

DISCUSSION

Results obtained in this study show *in vitro* relaxing effects of *C. pentandra* (FCP) hydroethanolic leaves extract, on various models of conductance and resistance arteries such as pig coronary artery, main superior mesenteric artery, thoracic aorta, and femoral and carotid arteries from rat. They also allowed characterization mechanisms of cellular and molecular signaling involved in the vasorelaxation induce by the extract.

Regarding vasorelaxant effects, our results show that they are strongly dependent on endothelium. Indeed, comparison of relaxing response of pig coronary artery rings, main superior mesenteric artery, thoracic aorta, and femoral and carotid arteries from rat, with or without endothelium shows a significant difference between both types of vessels with, respectively (102.30%, 67.88%, 49.64%, 48.51%, and 71.28%), of relaxation for vessels with endothelium against 0% for vessels without endothelium. These results are the same to those several studies showing the leading role of endothelium in vasorelaxation mechanisms induced by polyphenols plant.[20-23] Endothelial function plays an important role in control of the vascular tonus by means of vasorelaxant factors derived of endothelium such as NO, EDH, and PGI2.[20,24-26]

Inhibition of endothelial NO synthase by L-NA which induced a significant decrease of vasorelaxation demonstrating the key role played by NO in vasorelaxation caused by FCP. However, L-NA does not block totally relaxation caused by *C. pentandra* extract, suggesting as well as other ways of road marking could be involved.

Our results ruled out EDH implication in relaxation induced by FCP confirming as well as their contribution in vasorelaxation of big arterial trunks such as the aorta is low such as demonstrated by other works.[27,28] Indeed, EDH would, especially, be present in microvessels such mesenteric bed.[29] In these conditions, the only possible way of relaxation from endothelium stayed that involving COXs and PGI2. However, our results showed no significant reduction of relaxing response when the coronary arteries ring provided of endothelium is incubated with an inhibitor of COX such as indometacin. These results suggest that relaxations induce by FCP would involve specifically endothelial NO pathway. They were obtained in accordance with those of searches groups which showed that polyphenols extracted from some red wine are activators of enzymes involved in the synthesis and/or the liberation of relaxing factors.[30,31]

Moreover, relaxations are decreased by SOD analog membranaire and CAT (MnTMPyP and PEG-CAT). Relaxation generated by FCP is as well reduced by native CAT and N-acetyl cysteine which is an inductor of reduced glutathione showing that the induction of the relaxation involves a redox-sensitive event. Thus, the vascular relaxation provoked by FCP passes by the redox-sensitive pathway Src-PI3-kinase/Akt, as shows it the incubation by wortmannin and PP2, which remains the main pathway of activation of endothelial NO synthase by polyphenols plant.[16]

FCP can inhibit contractile response produced by U46619, KCl, and serotonin (5HT) on pigs coronary arteries ring in the presence of a functional endothelium. This inhibitive result is warned by L-NA in case where implication of NO.

Relaxation mechanisms of vascular smooth muscle by NO were widely described by numerous authors.[28,32,33] Our results show that this effect requires the activation of endothelial NO-synthase. NO diffuses in the endothelial smooth muscle cells. Its first effector is the guanylate cyclase, producing guanosine monophosphate cyclic. The latter causes activation of protein kinase C (PKC) which phosphorylate phosphatase of myosin light chains (myosin light chain phosphatase), decreasing the contracted state of cell because there is no more interaction actin-myosin. The phosphorylation of PKC is also responsible for a decrease of intracytosolic Ca^{2+} concentration available by favoring the recaptage of Ca^{2+} by sarcoplasmic endoplasmic reticulum Ca-ATPase. The mechanism leads to vascular relaxation.[33]

Phytochemical analysis of FCP gave indications about the nature of compounds involved in vasorelaxant activity. Indeed, total polyphenols dosage by Folin–Ciocalteu method showed that FCP is very rich in polyphenols, in particular, flavonoids, tannins, sterols, and triterpenes. It was recently reported that the plant polyphenols, in particular, those extracted from some red wine, could activate endothelial NOS of culture cells, obtained from pig coronary arteries, by a redox-sensitive mechanism leading to the activation of PI3 kinase/Akt.[34] The results obtained during our works confirmed these mechanisms. All results allow us to conclude that the crude extract of *C. pentandra* caused a vasorelaxation by an endothelial activation redox sensitive of eNOS through Src kinase and PI3 kinase/Akt.

CONCLUSION

C. pentandra holds vasorelaxants properties on pig coronary artery, thoracic aorta, the main superior mesenteric artery, and femoral and carotid artery from rat justifying its utilization in the treatment of the arterial HBP. This effect necessitates the presence of a functional endothelium and passes by the redox-sensitive pathway Src kinase and PI3-kinase/Akt.

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