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RESEARCH ARTICLE

Terminalia avicennioides causes redox-sensitive endothelium-dependent relaxation involving nitric oxide, prostacyclin, and endothelium-derived hyperpolarizing in porcine coronary artery and different conductance and resistance vessels from rats

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ABSTRACT

Background: Our work is part of the enhancement of plants of Senegalese pharmacopeia with cardiovascular aim, among which *Terminalia avicennioides*. **Aim and Objective:** The aim of this study was to determine whether a hydroethanolic bark extract of *T. avicennioides* (TAE) is able to induce a relaxant effect in porcine coronary, rat mesenteric artery, carotid, femoral arteries, and thoracic aorta and to determine the mechanism underlying this effect. **Materials and Methods:** Porcine coronary, rat mesenteric, carotid, femoral arteries, and thoracic aorta rings were suspended in organ chambers for the recording of changes in isometric forces. Rings with endothelium were incubated or not with L-Nitro Arginine to block nitric oxide (NO) synthase, MnTMPyP, polyethylene glycol catalase (CAT), an inhibitors of intracellular production reactive oxygen species; CAT, an inhibitor of extracellular reactive oxygen species; Wortmannin, an inhibitor of redox-sensitive pathway PI3 kinase/Akt; PP2 (4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo [3,4-d]pyrimidine), an inhibitor of Src kinase; apamin (APA) 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 (INDO), an inhibitor of cyclooxygenase before contraction with U46619 or phenylephrine (PE) and a concentration relaxation curve to TAE. In some experiments, the endothelium was removed before contraction with U46619 or PE and concentration relaxation to TAE. The phosphorylation level of Akt and eNOS was assessed by Western blot analysis. **Results:** The hydroethanolic bark TAE induces a vasodilatory effect in porcine coronary artery pre-contracted with U46619. This effect is endothelium-dependent and is mediated by NO, prostaglandin, and endothelium-derived hyperpolarizing. TAE also induces vasorelaxant effects in superior mesenteric arteries, carotid arteries, thoracic aorta, and femoral arteries from rat. **Conclusion:** TAE-induced sustaining phosphorylation of Akt and eNOS in endothelial cells. *T. avicennioides* induces vascular relaxation which can explain the benefic effect of this plant in the treatment of high blood pressure in Senegal.

KEY WORDS: *Terminalia avicennioides*; Vasorelaxant Effects; Porcine Coronary; Rat Artery

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INTRODUCTION

Cardiovascular diseases (CVD) constitute a plague which affects every year 17.1 million people, are 29% of the world mortality.[1] More than 82% of the deaths intervene in developing countries. Of all non-transferable affections, cardiovascular diseases are thus responsible for the most

important mortality in these countries. It considers that before 2030, about 23.6 million of people will die by cardiovascular disease.[2] In Africa, to take care of these diseases, medicinal plants represent practically the only therapeutic arsenal at the disposal of the populations. In spite of the progress realized in modern medicine in the field of chemistry, the African populations, in large numbers, turn to the medicinal plants. The estrangement, the rarity or non-existence health center in villages, not availability, and too high cost of pharmaceutical products are, among others, some factors which explain this appeal to the traditional medicine.[3] It is moreover reasons why the WHO encourages recourse to traditional medicine.[4] It is all the sense of our study that concerns *Terminalia avicennioides*, a plant of the Senegalese pharmacopeia which is used in the coverage of the arterial high blood pressure. This species belongs to the genre Terminalia which made the object of numerous studies attributing her several therapeutic properties among which of antihypertensive effects.[5-8] From then on, the objective of our study is to determine *ex vivo* the vasoactive properties of hydroethanolic crude extract barks of *T. avicennioides* in porcine coronary arteries. We also tested the same extract on various conductance and resistance vessels from rat. Mechanisms involved in these effects will be also characterized.

MATERIALS AND METHODS

Plant Material

The barks of *T. avicennioides* were collected in April 2015 in the forest of Malicounda (Department of Mbour, Senegal). They were identified in the botanic laboratory of IFAN (Intitut Fondamental d'Afrique Noir) of Cheikh Anta Diop University of Dakar. Voucher specimens were deposited at the herbarium of the university under No. IFAN55792. We proceeded to the drying of the drug during 15 days shielded from the light before its pulverizing. The powder so obtained was preserved in the ambient temperature (25–30°C), in-room aerated until it is routing in the Laboratory of Biophotonics and Pharmacology, Faculty of Pharmacy of University of Strasbourg (France) where the vascular reactivity experiments and western blot analysis were performed.

The barks powder (10 g) of *T. avicennioides* was macerated in 100 ml of an aqueous-ethanolic solution (60 ml of ethanol and 40 ml of water) in an Erlenmeyer flask. It was mixed for 24 h at room temperature. After this, the macerate was filtered on a funnel with cotton wool by gravity. The filtrate was collected and evaporated using a rotary evaporator under the following conditions: Temperature of the water bath 40°C, obtained by a vacuum pump Vacuubrand membrane. Crude bark extract was obtained after evaporation. This extract was directly frozen in liquid nitrogen before lyophilization. This step allowed to obtain a solid content to make the vascular reactivity tests and western blot analysis.

Phytochemical Screening

Phytochemical tests were conducted on hydroethanolic crude bark extract of *T. avicennioides* (TAE) to determine the presence of flavonoids, tannins, sterols, and terpenoids using standard protocols.[9,10]

Determination of Total Phenolic Contents

The total phenolic contents were determined in triplicate and expressed as mg gallic acid equivalents (GAE) using the Folin–Ciocalteu method.[11]

Chemical Material

L-Nitro Arginine (L-NA), indomethacin (INDO), apamin (APA), Tram-34, catalase (CAT), and polyethylene glycol CAT (PEG-CAT) were obtained from Sigma Chemical Co. (Saint Louis, MB, U.S.A). Wortmannin and the SOD mimetic Mn(III)tetrakis(1-methyl-4-pyridyl)porphyrin (MnTMPyP) were from Alexis Chemicals. U46619 (9,11-dideoxy-9α-methanoepoxy prostaglandin [PGI_2] $F_{2\alpha}$) and PP2 (4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo [3,4-d]pyrimidine) 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). The thoracic aorta, the main superior mesenteric artery, the carotid and femoral artery from rats anesthetized with phenobarbital (50 mg/kg, intraperitoneally), and the left circumflex coronary artery was 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. For some experiments, the endothelium was removed mechanically by rubbing the intimal surface of the rings by means of a notched clamp. The rings were subsequently suspended between two metal hooks in tanks isolated organ 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 connected to an isometric tension sensor which measures the variations. Following an equilibration period of 45 min to 1 h under a resting tension of 1 g for mesenteric, carotid, and femoral arteries, 2 g for thoracic aorta and 5 g for porcine coronary artery, after an equilibration period of 60 min, the rings were contracted with a Krebs solution containing 80 mM KCl to verify the integrity of the artery. After washout and a further 30 min equilibration period, rings of rat mesenteric, carotid, femoral arteries, and thoracic aorta were again contracted with PE (1 μM) and the

relaxation to acetylcholine (1 μM) was determined to test the integrity of the endothelium. Similarly, rings of porcine coronary arteries were contracted with the thromboxane mimetic U46619 (1–60 nM) to about 80% of the maximal contraction before the addition of bradykinin (0.3 μM) to check the presence of a functional endothelium. After washout and a 30 min equilibration period, rings were again contracted with U46619 for porcine coronary artery and PE for rat thoracic aorta, mesenteric, and carotid artery before a concentration-relaxation curve to TAE. In some experiments, rings were exposed to an inhibitor(s) for 30–45 min. The global component was defined as the relaxation obtained in the absence of inhibitors. The nitric oxide (NO)- plus EDHF-mediated relaxation was determined in the presence of indomethacin (10 μM) to rule out the formation of vasoactive prostanoids. The PGI_2-mediated component of relaxation was recorded in the presence of L-NA (300 μM) and Tram-34 plus APA (100 nM each) to rule out the formation of NO and endothelium-derived hyperpolarizing (EDH), respectively. Relaxations were expressed as a percentage of the contraction induced by PE or U46619.

In another set of experiments, cumulative contractile responses induced by U46619 (1 nM–100 nM), 5HT (10 nM–10 μM), and KCl (10 mM–80 mM) were obtained in the presence of TAE (1, 3, and 10 μg/ml) in the presence of a functional endothelium. Finally, TAE was incubated for both previous conditions in the presence of L-NA (300 μM), an NO synthase inhibitor; Tram-34 (10 μM) plus APA (100 nM), an inhibitor of small and intermediary conductance potassium channels calcium-dependent (SKCa, IKCa); and INDO (10 μM), an inhibitor of PGI_2.

Culture of Porcine Coronary Artery Endothelial Cells

Segments of porcine coronary arteries were flushed with PBS without calcium to remove the remaining blood. Thereafter, endothelial cells were isolated by collagenase treatment (type I, Worthington, 1 mg/ml for 15 min at 37°C), and cultured in culture dishes containing MCDB 131 medium (Invitrogen) supplemented with 15% fetal calf serum, penicillin (100 U/ml), streptomycin (100 U/ml), fungizone (250 μg/ml), and L –glutamine (2 mM) (all from Cambrex) and grown for 48–72 h. All experiments were performed with confluent cultures of cells used at first passage. Cells were exposed to a serum-free culture medium in the presence of 0.1% bovine serum albumin (Qbiogene) for 6 h before treatment.

Western Blot Analysis

The level of phosphorylation of Akt and endothelial eNOS was determined in cultured endothelial cells using Western blot analysis. After treatment, the endothelial cells were washed twice with PBS and then lysed in an extraction buffer of the following composition in mM: Tris/HCl 20 (pH 7.5; Qbiogene), NaCl 150, Na_3 VO_4 1, sodium pyrophosphate 10, NaF 20, okadaic acid 0.01 (Sigma), a tablet of protease inhibitor (Roche), and 1% Triton X-100 (Qbiogene). Total proteins (20 μg) were separated on 8% SDS-polyacrylamide (Sigma) gels at 70 V for 2.5 h. Separated proteins were transferred electrophoretically on to polyvinylidene difluoride membranes (Amersham) at 100 V for 120 min. Membranes were blocked with a blocking buffer containing 3% bovine serum albumin for p-Akt and p-eNOS, and I-block for p-Src, Tris-buffered saline solution (Biorad), and 0.1% Tween 20 (Sigma) for 1 h. For the detection of phosphorylated proteins, membranes were incubated with the respective primary antibody (p-Akt Ser473 and p-eNOS Ser1177, Cell Signaling Technology; dilution 1: 1000) overnight at 4°C. After washing, membranes were incubated with the secondary antibody (peroxidase-labeled anti-rabbit IgG, dilution 1:5,000; Cell Signaling Technology) at room temperature for 60 min. Prestained markers (Invitrogen) were used for molecular mass determinations. Immunoreactive bands were detected by enhanced chemiluminescence (Amersham). Ponceau staining was performed to verify the quality of the transfer and equal amounts of proteins in each lane. Densitometric analyses were performed with the software ImageJ.

Statistical Analysis

The results are expressed as means ± SEM of 6–8 experiments. Statistical significance was determined through a one-way analysis of variance (ANOVA) followed by Bonferroni's test or with Student's *t*-test for paired data as required. Statistical analysis was performed using GraphPad Prism version 6.01® for Windows (GraphPad Software, San Diego, Calif., USA). Values of $P < 0.05$ were considered statistically significant.

RESULTS

Phytochemical Analysis of Crude Hydroethanolic TAE Bark

Reagent-based phytochemical screening of the crude hydroethanolic TAE bark revealed the presence of flavonoids and tannins. However, sterols and triterpenes are not detected [Table 1]. The content in total phenolic compounds was 982.2 ± 3.2 mg of GAE/gram of extract [Table 2].

TAE Induces Endothelium-dependent Relaxations in Coronary Artery Rings

The addition of cumulative concentrations of TAE on isolated porcine coronary artery rings contracted with U46619-induced concentration-dependent relaxations in endothelium-intact but not in endothelium-denuded arteries [Figure 1a]. The endothelium-dependent relaxations started at concentrations greater than 0.3 μg/ml and reached a near-maximal value at 3 μg/ml (E_{max} = 100,16%). Relaxations to TAE were maximally affected (around 67.92% of inhibition of E_{max}) by L-NA (300 μM), an inhibitor of eNOS

[Figure 1b]; significantly affected (20.24% of inhibition of E_{max}) by INDO (10 μM), an inhibitor of cyclooxygenases [Figure 1c]; significantly affected (approx. 15,12% of inhibition of E_{max}) by the combination of Tram-34 (1 μM) plus APA (100 nM), two inhibitors of EDH-mediated responses [Figure 1d]; almost abolished (84% of inhibition of E_{max}) by the combination of L-NA with INDO and Tram-34 plus APA [Figure 2b]. Altogether, these findings indicate that TAE causes endothelium-dependent relaxations, mainly dependent on NO component and also, EDH and PGI_2 sensitive component.

Table 1: Class of phytochemical constituents of crude hydroethanolic TAE bark

Compounds screened for	TAE
Tannin	++
Flavonoid	+++
Sterol and triterpene	–

The test was positive (+) when compound screened for was detected, negative (−) when it was not found. *T. avicennioides: Terminalia avicennioides*, TAE: Extract of *T. avicennioides*

Table 2: Concentration of total phenols in *T. avicennioides* extracts by Folin–Ciocalteu assay

TAE	Total polyphenolic contents (mg GAE/g)
	982.2±3.2

Results are expressed as mg of gallic acid equivalent (GAE) per gram of extract under the form of mean±SEM (*n*=3). *T. avicennioides: Terminalia avicennioides*, TAE: Extract of *T. avicennioides*

Role of ROS, Src Kinase, and the PI3-Kinase/Akt Pathway TAE-induced Relaxations

Recent findings indicate that polyphenolic compounds can induce NO-mediated relaxations in porcine coronary arteries by a mechanism involving the endothelial production of ROS and activation of the PI3-kinase/Akt and Src kinase pathway[12-15] leading to eNOS phosphorylation. The consequent activation of eNOS through this pathway has been found to occur even in a Ca^{2+}-free medium.[16] Therefore, experiments were performed using MnTMPyP (100 μM), a membrane-permeant SOD mimetic, CAT (500 U/ml), and PEG-CAT (500 U/ml), a membrane-permeant CAT mimetic, to determine whether a ROS signaling pathway is involved in TAE-induced relaxations.

The relaxations induced by TAE (0.1–3 μg/ml) were markedly reduced by the membrane-permeant SOD mimetic MnTMPyP, by the cell-permeable PEG-CAT and native CAT (approx. 100%; 99%; and 97% of inhibition of E_{max}, respectively) [Figure 2a and b].

Moreover, it has been shown 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] Experiments were performed, therefore,

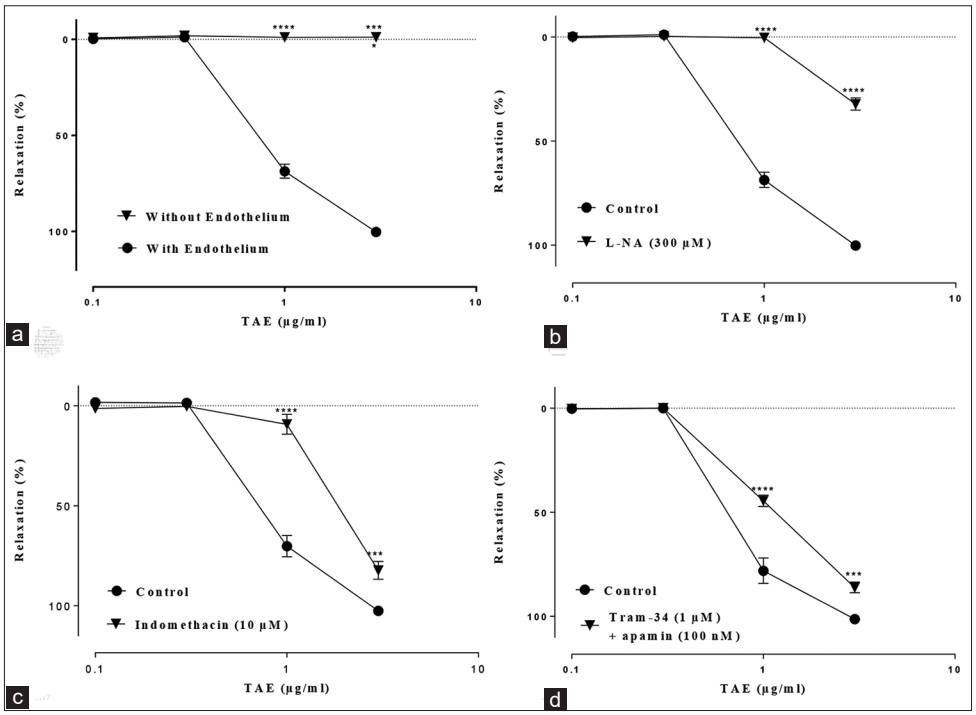

Figure 1: Characterization of extract of *Terminalia avicennioides* (TAE)-induced relaxations in isolated porcine coronary artery rings. Intact and endothelium-denuded rings were contracted with U46619 before the addition of cumulative concentrations of TAE. (a) Endothelium-dependent relaxations induced by TAE. (b) Effect of L-NA (300 μM). (c) Effect of indomethacin (10 μM) on TAE-induced relaxations in coronary artery rings with endothelium. (d) Relaxant effect of TAE in the presence of apamin (APA, 100 nM) plus Tram-34 (1 μM) in intact arteries. Results are shown as means ± SEM of 6 different experiments. ****$P < 0.0001$ for inhibitory effect versus control

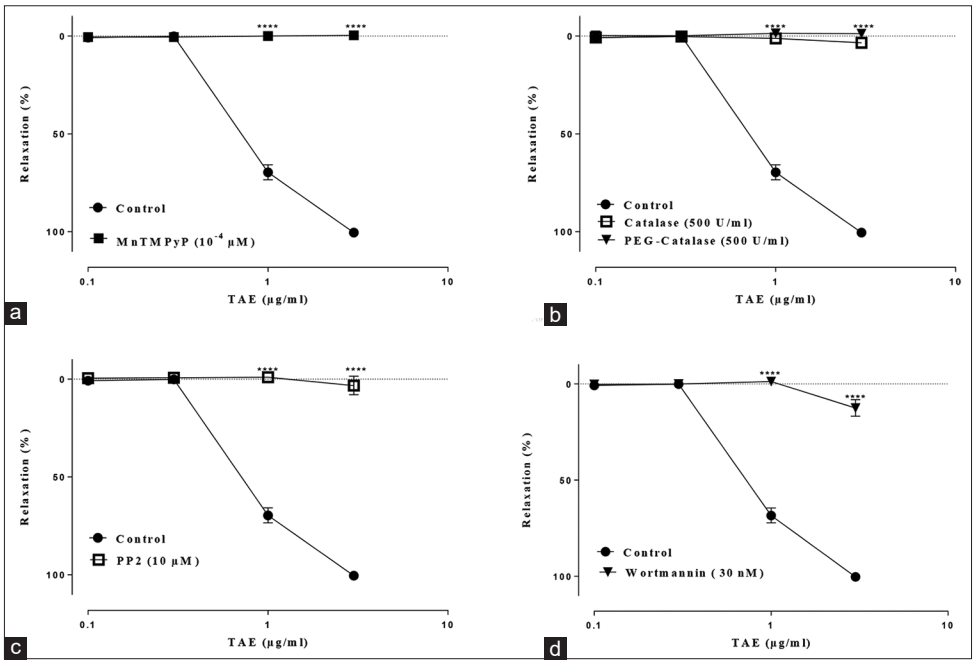

Figure 2: Role of the redox-sensitive Src kinase and the PI3-kinase/Akt pathway in extract of *Terminalia avicennioides* (TAE)-induced endothelium-dependent relaxations. Coronary artery rings with endothelium were incubated with MnTMPyP (100 µM), a cell-permeable SOD mimetic (a), native catalase (CAT, 500 U/ml) or polyethylene glycol CAT (PEG-CAT, 500 U/ml), a membrane-permeant analog of CAT (b), the Src kinase inhibitor PP2 (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 ± SEM of 6 different experiments. ****$P < 0.0001$ for inhibitory effect versus control

to determine the role of the Src kinase/PI3-kinase/Akt pathway in relaxations to TAE. Inhibition of Src kinase with PP2 (10 µM) and the PI3-kinase by wortmannin (30 nM) abolished relaxations to TAE (98% and 88% of inhibition, respectively) [Figure 2c and d].

TAE Causes the Redox-Sensitive Activation of Src with Subsequent PI3-Kinase/Akt-dependent Phosphorylation of eNOS

To better characterize the signaling pathway involved in eNOS activation in response to TAE, levels of phosphorylated Akt and eNOS were assessed in endothelial cells by Western blot analysis. TAE (1–100 µg/ml, 10 min) evoked the concentration-dependent phosphorylation of Akt at Ser473 and eNOS at Ser1177 in endothelial cells [Figure 3].

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

Exposure of coronary artery rings to TAE (1, 3, 10 µg/ml) during 30 min before the addition of cumulative concentrations of U46619 (1 nM–100 nM), 5HT (10 nM–10 µM), and KCl (10 mM–80 mM) significantly reduced contractions in rings with endothelium [Figure 4a-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 4d].

TAE Induces Relaxations in Different Conductance and Resistance Arteries Rings from Rats

The addition of cumulative concentrations of TAE on isolated thoracic aorta, the main superior mesenteric arteries, ad femoral, and carotid arteries rings from rats contracted with PE-induced concentration-dependent relaxations in endothelium-intact preparations [Figure 5]. TAE effects are more marked on the superior mesenteric artery (83.62% at 10 µg/ml) against 78.64% of relaxation on the thoracic aorta, 61.39% on carotid artery and 50.82% on femoral arteries ring from rats.

DISCUSSION

The results obtained during this work demonstrate the relaxing effects of hydroethanolic barks TAE, on diverse models of vessels isolated to pig coronary artery; thoracic aorta from rat, the main superior mesenteric artery, the femoral, and carotid artery from rat. However, more interesting still, they allowed the characterization of the mechanisms of the cellular and molecular pathway involved in the vasorelaxation induced by this plant of Senegalese and African pharmacopeia.

Concerning vasorelaxant effects, the analysis of our results showed that they are strongly dependent on endothelium on all the used arteries models. Indeed, the comparison of the relaxing response of pig coronary artery rings, rat thoracic aorta, the main superior mesenteric artery from rat, femoral,

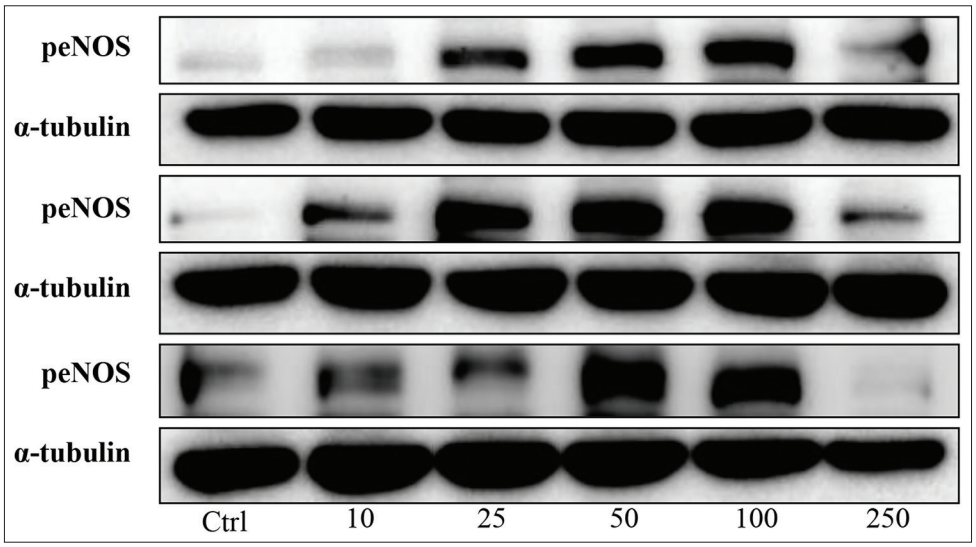

Figure 3: Extract of *Terminalia avicennioides* causes concentration-dependent phosphorylation of Akt at Ser473 and eNOS at Ser1177 in cultured porcine coronary artery endothelial cells. Results are shown as means ± SEM of 3 different experiments. ****$P < 0.0001$ for inhibitory effect versus control

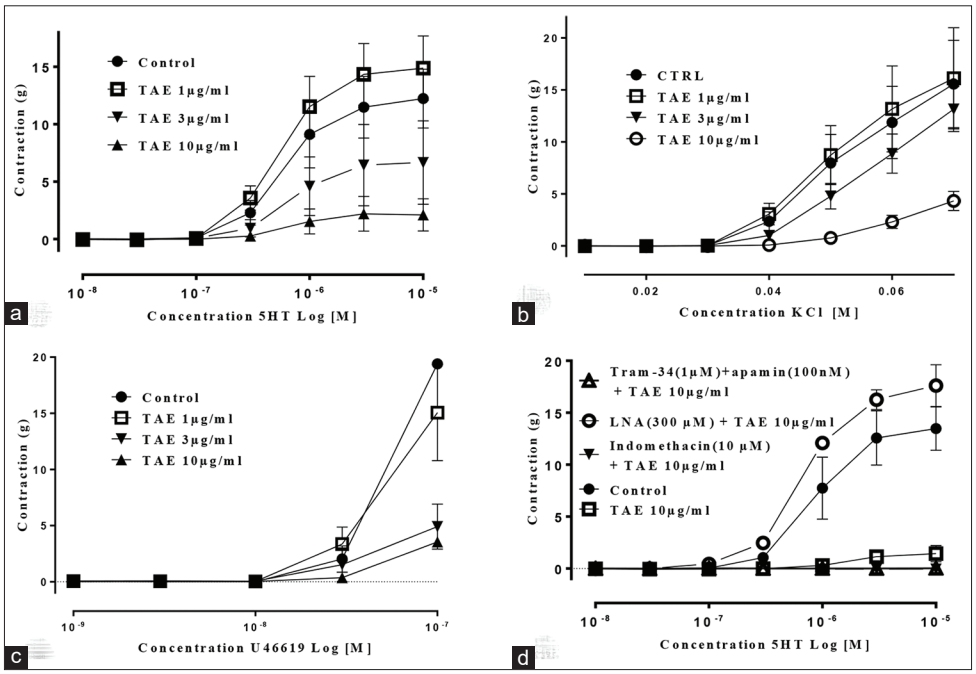

Figure 4: Effect of extract of *Terminalia avicennioides* (TAE) on contractile responses in coronary artery rings with endothelium. The graph shows contractile curves to 5HT, KCl, and U46619 assessed either in the presence of TAE (1, 3, 10 µg/ml) (a-c). The graph shows contractile curves to 5HT assessed either in the presence or absence of TAE (10 µg/ml), with or without L-NA (300 µM, 30 min; Tram-34 1 µM plus apamin 100 nM, 30 min; indomethacin 10 µM) (d). Results are shown as means ± SEM of 8 different experiments

and carotid artery from rat with and without endothelium shows a significant difference between 2 types of vessels with, respectively, (100.16%, 78.64%, 83.62%, 50.82%, 61.39% of relaxation for vessels with endothelium versus 0% for vessels without endothelium).

These results go to the same sense as those brought reported by numerous studies demonstrating the dominant role of vascular endothelium in the mechanisms of vasorelaxation induced by plant polyphenols.[19-22,24,39]

It is generally admitted that endothelium-dependent vasorelaxation is a matter of three major relaxing factors, worth knowing NO, prostacyclin (PGI_2), as well as EDH. These various relaxing factors are respectively produced by the endothelial NO-Synthase and the cyclooxygenase (COX) and a source was not yet determined for EDH.[23-25]

Inhibition of eNOS and COX respectively by L-NA and indometacin during our experiments causes a significant loss of vasorelaxation, so demonstrating the role of NO and PGI_2

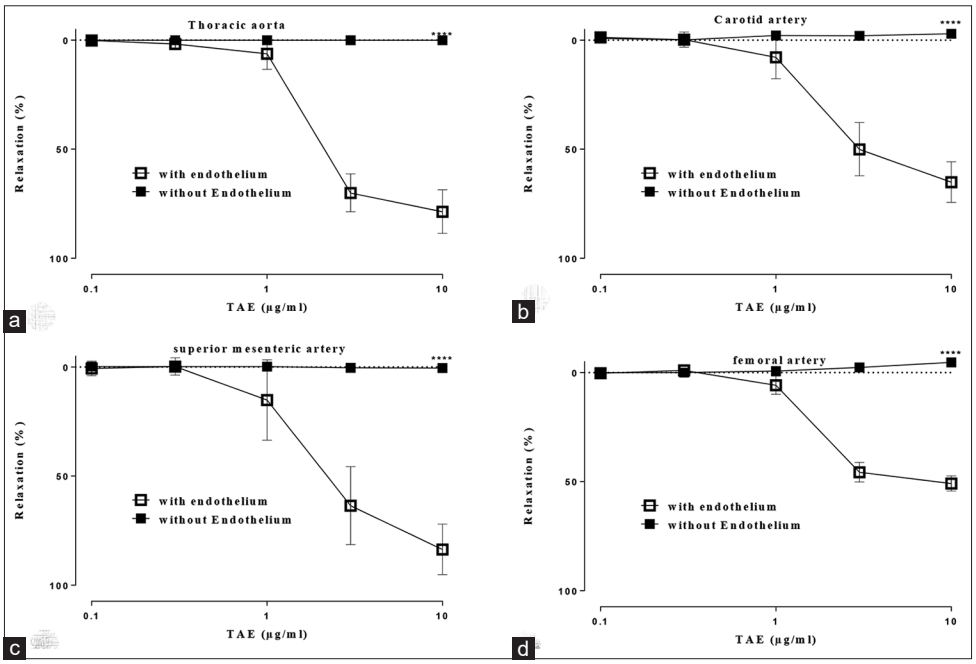

Figure 5: Characterization of extract of *Terminalia avicennioides* (TAE) induced relaxations in the thoracic aorta, the main superior mesenteric artery, the carotid, and femoral artery from rats. Rings with and without endothelium were contracted by phenylephrine before the addition of cumulative concentrations of TAE (0.1 to 10µM). (a) Relaxation effects induced by TAE in intact thoracic aorta. (b) Relaxation effects induced by TAE in carotid artery with endothelium. (c) Relaxation effects induced by TAE in intact main superior mesenteric artery. (d) Relaxation effects induced by TAE in intact femoral artery from rats. Results are shown as means ± SEM of 6 different experiments. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$

in the vasorelaxation induced by TAE on pig coronary artery rings.

However, as well L-Na as indometacin does not inhibit the relaxing response of TAE on this model, suggesting as well as other ways of relaxation involving other enzymes and/or mechanisms and pathway marking would be involved.

Contribution of EDH in vasorelaxation of big arterial trunks such as coronary artery and the thoracic aorta is described as weak by numerous authors.[26-28] Indeed, EDH would especially be present in microvessels such as the mesenteric bed and carotid. However, use of the combination tram-34 plus APA who are inhibitors of intermediary conductance potassium channels calcium-dependent (IKCa) and small conductance (SKCa) calcium-dependent has caused a significant reduction of the relaxation induced by TAE on pigs' coronary arteries rings. These results suggest that the relaxations induced by TAE would involve the NO pathway and EDH.

The relaxation mechanisms of vascular smooth muscle by NO were widely described by numerous authors.[29,30-32,39] Our results show that this effect requires the activation of endothelial NO-Synthase. They are in accordance with those obtained by groups of researches which showed that polyphenols extracted from some red wine are activators of enzymes involved in the synthesis and/or the liberation of relaxing factors.[19,33]

Besides, the relaxations are reduced by a superoxide dismutase analog and CAT (MnTMPyP and PEG-CAT). The relaxation induced by TAE is also reduced by native CAT and NAC which is an inductor of the reduced glutathione, indicating that the induction of the relaxation involves a redox-sensitive event. Hence, the vascular relaxation induced by TAE 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.[34]

In endothelial cells, training of superoxide anion can activate Src-PI3-kinase/Akt pathway leading to phosphorylation of eNOS at Ser1177 in response to polyphenols.[35] This pathway can be also activated in response to several physiological stimuli, including estrogens, strengths of cutting of blood, growth factor of vascular endothelial cell, H_2O_2, and high-density lipoprotein.[36,37] TAE caused activation of redox-sensitive pathway PI3-kinase/Akt as indicated by phosphorylation-dependent on the concentration of Akt at Ser473 and eNOS at Ser1177, on endothelial cells culture.

TAE is able to inhibit contractile response induced by U46619, KCl, and serotonin (5HT) on pigs' coronary arteries rings in the presence of a functional endothelium. This inhibitive effect is warned by L-NA where the implication of NO.

NO diffuse in endothelial smooth muscle cells. Its first effector is the guanylate cyclase, producing cyclic GMP.

GMPc will activate PKC which phosphorylates phosphatase of myosin light channels (MLCP), decreasing the contraction of cell because there is no actin-myosin interaction anymore. Phosphorylation of PKG is also responsible for a decrease of intracytosolic Ca^{2+} concentration by favoring recaptage of Ca^{2+} by Sarcoplasmic Endoplasmic reticulum Ca-ATPase. The mechanism leads to vascular relaxation.

Phytochemical analysis of TAE gave indications to the nature of compounds involved in vasorelaxant activity. Indeed, the dosage of total polyphenols by the Folin–Ciocalteu method showed that TAE is very rich in polyphenols in particular flavonoids and tannins. It was brought back recently that polyphenols plant, in particular, those extracted from red wine, could activate NOS of endothelial cells culture, obtained from pig coronary arteries, by a redox-sensitive mechanism leading to activation of PI3K/Akt.[38] The results obtained during our works confirmed these mechanisms.

CONCLUSION

All results allow us to conclude that the crude TAE induces a vasorelaxation on coronary arteries and different resist if and conductance model by the following mechanisms

- An endothelial activation redox-sensitive of NOS through Src Kinase and PI3K/Akt
- An activation of intermediary and small conductance potassium channels hyperpolarizing calcium-dependent (IKCa and SKCa)
- An endothelial activation of COX.

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