Original Article

The Methanolic Fraction of *Combretum Glutinosum* bark extract (MSCG01) Induces Endothelium-Dependent Relaxation Through Nitric Oxide (No) Involvement in Porcine Coronary Arteries

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Abstract

Background: The bark of *Combretum glutinosum* Perr. (Combretaceae) is traditionally used in the treatment ofarterialhigh blood pressurein Senegal. The aim of this study was to determine the vasorelaxant effect of a methanolic fraction of Combretum glutinosum bark extract (MSCG01) on isolated porcine coronary arteries and to investigate the underlying mechanism.

Methods: Rings of porcine coronary arteries were suspended in organ chambers to record changes in isometric tension. Rings with intact endothelium were incubated in the presence or absence of various inhibitors, including L-nitro-arginine (L-NA), an inhibitor of endothelial nitric oxide synthase (eNOS), UCL, an inhibitor of calcium-dependent small conductance potassium channels (SKCa), and Tram-34, an inhibitor of calcium-dependent intermediate conductance potassium channels (IKCa). Indomethacin (INDO), an inhibitor of cyclooxygenases, was also used. Thirty minutes after incubation with an inhibitor, the rings were contracted with U46619, a thromboxane A2 mimetic, and subsequently relaxed with increasing concentrations of MSCG01. In some experiments, the endothelium was removed before contraction with U46619 and relaxation with MSCG01.

Results: MSCG01 induces 100% relaxation at 10 µg/ml in endothelium-intact artery rings pre-contracted with U46619. The MSCG01-induced an endothelium-dependent relaxation mediated by nitric oxide (NO), but not by endothelium-derived hyperpolarizing factors (EDH) or prostanoids.

Conclusions: *Combretum glutinosum* induces vasodilation which may contribute to explain its antihypertensive effect and its use in traditional African medicine.

Keywords: *Combretum glutinosum*, vasorelaxant effects, porcine coronary.

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Introduction

Hypertension is a major public health concern, affecting over one billion people worldwide and constituting a leading cause of mortality [1, 2]. Despite the availability of numerous treatments, its prevalence continues to rise [3]. Hypertension is recognized both as a standalone pathology and as a significant risk factor for other vascular, cardiac, cerebral, and renal diseases, contributing to an estimated 7.6 million deaths globally [2, 4]. By 2025, the World Health Organization (WHO) projects that approximately 1.5 billion people will be affected, driven by an aging population and shifts in lifestyle. Hypertension is not confined to a specific population or ethnicity; however, individuals in low-income countries face higher risks (31.5%) compared to those in industrialized nations (28.5%) [1].

The management of hypertension requires continuous monitoring and lifelong treatment. However, the high cost of pharmaceuticals has led to the use of herbal medicine by nearly 85% of the population in underdeveloped countries [5]. *Combretum glutinosum* is a medicinal plant widely used in Africa by traditional practitioners for the treatment of various ailments [6]. In Senegal, this plant is traditionally used to lower blood pressure in individuals with hypertension, but to date, no scientific evidence has substantiated its efficacy. In this context, we aimed to investigate the vasorelaxant properties of the methanolic fraction of *Combretum glutinosum* bark powder using a porcine coronary artery model and to characterize the underlying mechanisms.

Materials and Methods

Plant Material

Barks of *Combretum glutinosum*from the *Combretaceae* family were collected in may 2022 at the botanical garden of the Faculty of Medicine, Pharmacy, and Odontology at the Cheikh Anta Diop University of Dakar (Senegal). The barks were identified at the botanic laboratory of IFAN (Institut Fondamental d'Afrique Noire) at Cheikh Anta Diop University of Dakar. Voucher specimens were deposited at the university herbarium under No. IFAN58192. The barks were dried for 15 days, protected from light, before being pulverized. The resulting powder was stored at room temperature (25-30°C) in a well-ventilated room until it was transported to the Laboratory of Translational Cardiovascular Medicine UR 3074, FMTS, Strasbourg, France, where the vascular reactivity experiments were conducted.Extraction and fractionation were carried in laboratory of pharmacognosia of Faculty of Medicine of University Cheikh Anta Diop, Dakar, Senegal [7].

Chemical Material

Nω-nitro-L-arginine (L-NA), indomethacin (INDO), UCL, and Tram-34 were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). U46619 (9,11-dideoxy-9α-methanoepoxy prostaglandin F2α) was purchased from Calbiochem, and bradykinin was obtained from Cayman Chemical (Ann Arbor, MI, U.S.A.).

Vascular Reactivity Studies

This study conforms to the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996). It was conducted between April and June 2023 at the Laboratory of Translational Cardiovascular Medicine, UR 3074, FMTS, Strasbourg, France. The left circumflex coronary artery was removed and carefully cleaned of fat and connective tissue in a physiological Krebs bicarbonate solution at 4 °C. The artery was then cut into rings of 3 to 4 mm in length. For some experiments, the endothelium was removed mechanically by rubbing the intimal surface of the rings with a notched clamp. The rings were subsequently suspended between two metal hooks in 10 ml isolated organ baths, 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 to measure the variations in force.

To measure changes in isometric tension, each ring was maintained under a basal tension of 5 g. After an equilibration period of 60 minutes, the rings were contracted with Krebs solution containing 80 mM KCl to verify artery integrity. Following washout and an additional 30-minute equilibration period, rings of porcine coronary arteries were contracted with the thromboxane A_2 mimetic U46619 (1-60 nM) to approximately 80% of the maximal contraction before adding bradykinin (0.3 µM) to check for the presence of a functional endothelium. Vessels were considered to have a functional endothelium if bradykinin induced greater than 90% relaxation. The vessels were then washed three times with Krebs solution and incubated separately with or without various inhibitors of endothelial vasorelaxant factors (L-NA, 300 µM; indomethacin, 10 µM; UCL, 100 nM; Tram-34, 1 µM) for 30 minutes before contracting with U46619. The sustained contractions were then assessed by adding the *Combretum glutinosum* barkss powder extract (MSCG01) in a cumulative manner to construct a relaxation-concentration curve.

Statistical Analysis

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 test for paired data as required. Statistical analysis was performed using Graph*Pad. Prism version 6.01* ® for Windows (GraphPad Software, San Diego, Calif., USA). Values of $p < 0.05$ were considered statistically significant.

Results

Role of endothelium in the vascular effects of MSCG01: MSCG01 induced relaxation of pig coronary having a functional endothelium and pre-contracted U46619 (Figure 1). The endothelium-dependent relaxations started at concentrations greater than 0.3 µg/ml and reaches a maximal value close to 10 µg/ml (E_{max} = 98,82 %).

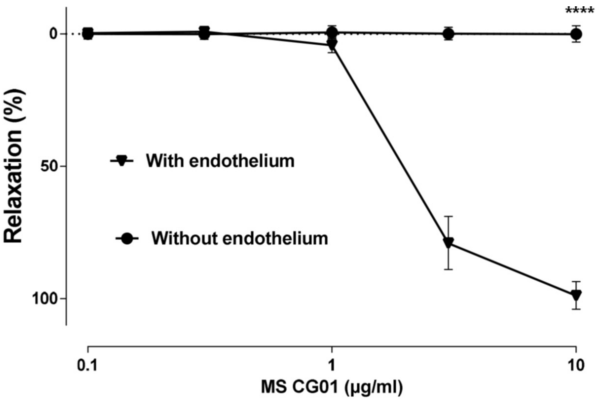

Figure 1: Effect-concentration curves of MSCG01 in isolated porcine coronary artery pre-contracted with U46619, with or without functional endothelium. Results are shown as means ± SEM of 6 different experiments.**p < 0.0001 for inhibitory effect versus control.**

Role of nitric oxide in the vasorelaxant effects of MSCG01: Incubation for 30 minutes of L-NA (300 µM), inhibitor of endothelial NO synthase, caused a significant reduction in the endothelium-dependent vasorelaxation induced by MSCG01 (Figure 2).

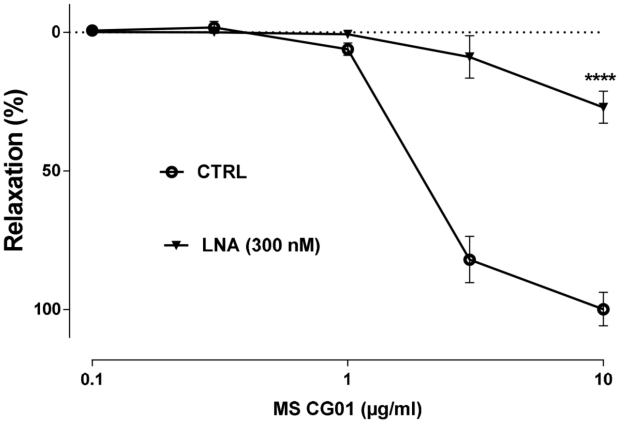

Figure 2: Effect-concentration curves of MSCG01 in isolated porcine coronary artery pre-incubated with L-NA (300 µM) for 30 minutes' incubation before addition of U46619. Results are shown as means ± SEM of 6 different experiments. **** p < 0.0001 for inhibitory effect versus control.

Role of prostacyclin in the vasorelaxant effects of MSCG01: Presence of indomethacin (10 µM) for 30 min, an inhibitor of the synthesis of prostacyclin,

did not affect significantly the endothelium-dependent vasorelaxation induced by MSCG01 (Figure 3).

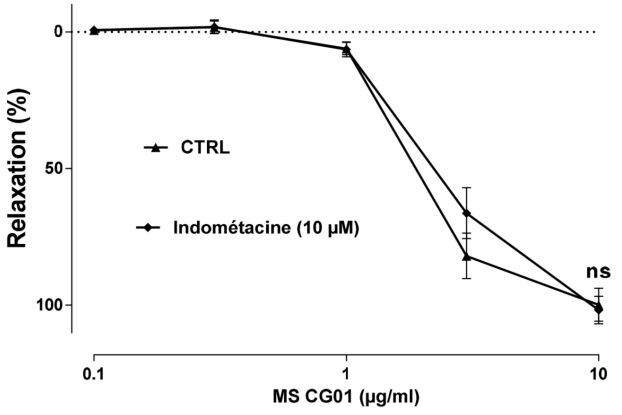

Figure 3: Effect-concentration curves of MSCG01 in isolated porcine coronary artery pre-incubated with indometacin (10 µM), for 30 minutes' incubation before addition of U46619. Results are shown as means ± SEM of 6 different experiments.n= 6

Role of EDHF in the vasorelaxant effects of MSCG01: Presence of UCL (100 nM) and TRAM (100 nM) for 30 min, an inhibitor of EDHF did not affect significantly the endothelium-dependent vasorelaxation induced by MSCG01 (Figure 4).

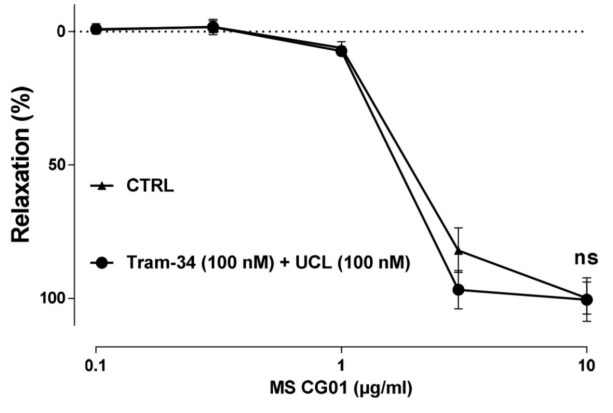

Figure 4: Effect-concentration curves of MSCG01 in isolated porcine coronary artery pre-incubated with UCL (100 nM) + TRAM (100 nM) for 30 minutes' incubation before addition of U46619. Results are shown as means ± SEM of 6 different experiments.n = 6

All these results show that MSCG01 induces endothelium-dependent relaxation via the production of nitric oxide (NO)

Discussion

The present study shows that methanol fraction bark extract of *Combretum glutinosum* (MSCG01) is a powerful inducer of relaxation dependent on endothélium involving eNOS pathway.

Our findings reveal that the vasorelaxant effects are highly dependent on the presence of endothelium. A comparison of the relaxation responses in porcine coronary artery rings, with and without endothelium, shows a marked difference: 98.82% relaxation in endothelium-intact vessels versus 0% in endothelium-denuded vessels. These findings are consistent with previous research underscoring the essential role of the endothelium in vasorelaxation mechanisms triggered by plant polyphenols [8, 9, 10, 11]. Endothelial function is pivotal in regulating vascular tone via endothelium-derived vasorelaxant factors such as nitric oxide (NO), endothelium-derived hyperpolarizing factor (EDH), and prostacyclin (PGI_2) [12, 13, 14, 15]. The inhibition of endothelial NO synthase by L-NA, resulting in a significant attenuation of vasorelaxation, highlights the central role of NO in the vasorelaxation induced by MSCG01. However, the incomplete blockade of relaxation by L-NA suggests the involvement of additional mechanisms.

Our results exclude the involvement of endothelium-derived hyperpolarizing factor (EDHF) in the relaxation induced by MSCG01, reinforcing the notion of EDH's limited role in the vasorelaxation of large arterial trunks such as coronary arteries, as demonstrated by previous studies [16, 17, 18]. EDHF is primarily active in microvessels, such as those in the mesenteric bed [19]. Under these circumstances, the remaining pathway for endothelium-dependent relaxation would likely involve cyclooxygenases (COX) and prostacyclin. However, our findings revealed no significant reduction in relaxation when coronary artery rings with intact endothelium were incubated with the COX inhibitor indomethacin. This suggests that the relaxation induced by MSCG01 predominantly involves the endothelial nitric NO pathway. These results are consistent with studies showing that polyphenols extracted from red wine can activate enzymes involved in the synthesis and/or release of vasorelaxant factors [20, 21].

Relaxation mechanisms of vascular smooth muscle mediated by NO have been extensively described in the literature [17, 18, 22, 23]. Our results indicate that this effect necessitates the activation of endothelial NO synthase. NO diffuses into the smooth muscle cells of the endothelium, where its primary effector is guanylate cyclase, which produces cyclic GMP (cGMP). cGMP activates protein kinase G (PKG), leading to the phosphorylation of myosin light chain phosphatase (MLCP). This phosphorylation decreases the contraction of smooth muscle cells by inhibiting the actin-myosin interaction. Additionally, PKG activation reduces intracellular Ca^{2+} levels by promoting the reuptake of Ca^{2+} through the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA). This cascade of events results in vascular relaxation [23].

Phytochemical analysis of MSCG01 has demonstrated that it is rich in polyphenols, including flavonoids and tannins **(7)**. Recent studies have shown that plant polyphenols, particularly those derived from red wine, can activate endothelial nitric oxide synthase (eNOS) in cultured pig coronary artery cells via a redox-sensitive mechanism involving the activation of PI3K/Akt signaling [24, 25]. Our findings are consistent with these mechanisms. Collectively, our results suggest that methanol fraction bark extract of *Combretum glutinosum* induces vasorelaxation through the redox-sensitive activation of endothelial eNOS.

Conclusion

Combretum glutinosum possesses vasorelaxant properties on porcine coronary arteries, supporting its potential use in the treatment of arterial hypertension. This effect requires the presence of a functional endothelium and is mediated through the redox-sensitive eNOS pathway.

Conflict of Interest: The authors declare that they have no conflict of interest.

Source of Funding: This study is funded by the state of Senegal through the «study trip» program of Cheikh Anta Diop University in Dakar

Ethical Clearance: All manipulations were carried out after the approval of the ethics committee of the Cheikh Anta Diop University of Dakar (UCAD), at the laboratory of pharmaceutical physiology of the faculty of medicine, pharmacy and dentistry of the said university.

Limitations of study and future research recommendations: The relatively short duration of the research stay is a major limitation. This did not allow to demonstrate the involvement of the redox sensitive pathway SrcKinase/PI3kinase/AKT. The next step will be to confirm in vitro results on an in vivo model in hypertensive rats.

References

1. Ribon A et al.« Hypertension artérielle, dysfonction endothéliale et stress oxydant dans un modèle animal d'apnée du sommeil: rôle protecteur de l'estradiol», Université Laval et Université Claude Bernard Lyon 1, Québec, Canada et Villeurbanne,France, 2019.
2. Macia E, Duboz P, Gueye L. «L'hypertension artérielle à Dakar : prévalence, connaissance, traitement et contrôle », *Bull. Société Pathol. Exot. 2015*, vol. 108, nº 1, p. 4956, doi: 10.1007/s13149-014-0369-2.
3. Dib A et al.« Programmation foetale du système vasculaire en réponse à l'exposition in utero au diabète maternel chez le rat », PhD Thesis, Université d'Angers, 2016. Consulté le 8 avril 2024. [En ligne]. Disponible sur: https://univ-angers.hal.science/tel-02459311/
4. Sarkissian SD et al. « Contrôle pharmacologique de l 'apoptose cardiovasculaire dans l'hypertension : Implication de la voie de l'angiotensine et de la correction de la dysfonction endothéliale. », Université de Montréal, 2003. [En ligne]. Disponible sur: https://papyrus.bib.umontreal.ca/xmlui/bitstream/handle/1866/15036/Der_Sarkissian_Shant_2003_these.pdf
5. Kattouf J*et al*.« Effet antihypertenseur des feuilles d'Inula viscosa », *Phytothérapie*, 2009, vol. 7, nº 6, p. 309312, doi: 10.1007/s10298-009-0517-1.
6. Tine Y*et al*.« Revue des usages traditionnels, composition chimique et propriétés pharmacologiques de *Combretum glutinosum* (Combretaceae): Review of traditional uses, chemical composition and pharmacological proprieties of *Combretum glutinosum* (Combretaceae) », *Int. J. Biol. Chem. Sci.*, 2023 vol. 17, nº 6, p. 24752489, doi: 10.4314/ijbcs.v17i6.27.

7. Zougori KADet al. Evaluation des activités aniti-inflammatoire et analgesique de l'etrait methanolique d'ecorce de combretum glutinosum(combretaceae) riche en flavonoides et tanins [These Pharmacie 2023; 17]. [FMPO]: Universite Cheikh Anta Diop de Dakar.
8. Sene M., Kane MO, Sene M, Barboza FS, Sow AK, Diaw M, Ba F, Sarr M and Diallo AS. *Adansonia Digitata* Causes Redox-Sensitive Endothelium-Dependent Relaxation Involving NO in Porcine Coronary Artery. *J Phys Pharm Adv*, 2026, 6(6): 880-886. DOI: 10.5455/jppa.20160418115531
9. Furchgott, RF et al. Role of endothelium in reponses of vascular smooth muscle. *Circ Res; 1983, 53:557-73.*
10. Stoclet JC, Kleschyov A, Andriambeloson E, Diebolt M, Andriantsitohaina R. Endothelial no release caused by red wine polyphenols. *J Physiol Pharmacol; 1999, 50, 535-540.*
11. Wallerath T, Deckert G, Ternes T, Anderson H, Li H, Witte K, Forstermann U.Resveratrol, a polyphenolic phytoalexin present in red wine, enhances expression and activity of endothelial nitric oxide synthase. *Circ. 2002; 106, 1652-1658.*
12. Vanhoutte PM et al. Endothelium-derived free radicals:for worse and for better. *J Clin Invest, 2001; 707, 23-25.*
13. Vanhoutte PM et al. How to assess endothelial function in human blood vessels. *J Hypertens, 1999; 17: 1047-58.*
14. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature, 1980; 288: 373-6.*
15. Gauthier KM, Edwards EM, Falck JR, Reddy DS, Campbell W. 14,15 epoxyeicosatrienoic acid represents a transferable endothelium-dependent relaxing factor in bovine coronary arteries. *HTA, 2005; 45: 666- 71.*
16. De Moura RS, Miranda DZ, Pinto AC, Sicca RF, Souza MA, Rubenich LM,Carvalho LC, Rangel BM, Tano T, Madeira SV, Resende AC. Mechanism of theendothelium dependent vasodilation and the antihypertensive effect of Brazilian red wine. *JCardiovasc Pharmacol, 2004; 44(3):302-9.*
17. Kane MO, Anselm E, Rattmann YD, Auger C, Schini-Kerth VB. Role of gender and estrogen receptors in the rat aorta endothelium-dependent relaxation to red wine polyphenols. *Vascul Pharmacol, 2009; 51(2-3): 140-6.*
18. Sene M, Diouf I, Toure M, Auger C, Vonthron-Senecheau C, SarrM, Schini-KerthV, Kane MO. Vasoactive properties of *Ceiba pentandra* in porcine coronary artery and different conductance and resistance vessels from rats: A role of nitric oxide. *National Journal of Physiology, Pharmacy and Pharmacology, 2019* ;vol (9), issue (10).DOI:10.5455/njppp.2019.9.0308007082019
19. Dal-Ros C, Bronner C, Schott, Kane MO, Chataigneau M, Schini-Kerth VB, Chataigneau T. Angiotensin II-Induced Hypertension Is Associated with a Selective Inhibition of Endothelium-Derived Hyperpolarizing Factor-Mediated Responses in the Rat Mesenteric Artery. *J Pharmacol Exper Therap, 2009; 328:478–486*
20. Diallo AS, Sarr M, Mostafai A, Pricci M, Andrahtsitohaina R. Cognac Polyphenolic Compounds increase Bradykinin-induced Nitric Oxide production in endothelial cells. *PhysioLResearch, inpress, 2007.*
21. Fitzpatrick DF, Fleming RC, Bing B, Maggi DA, O'Mailey RM. Isolation andcharacterization of endothelium-dependent vasorelaxing compounds from grape seeds. *J Agric Food Chem, 2000; 48:6384-90*
22. Moncada S, Radonski MW, Palmer RMJ. Endothelium-derived relaxing factor, identification as NO and role in the control of vascular tone and platelet function. *Biochem. Pharmacol, 1988; 37:2495-501*
23. Rapoport RM, Diaznin MB, Murad F. Endothelium-dependent relaxation in rat aortamay be mediated through cyclic GMP-dependent protein phosphorylation. *Circ Res. 1983;52(3):352-7.*
24. Ndiaye M, Chataigneau T, Chataigneau M, Schini-Kerth VB. Red wine polyphenols induce EDHF-mediated relaxations in porcine coronary arteries through the redox-sensitive activation of the PI3-kinase/Akt pathway. *Br J Pharmacol, 2004; 142 : 1131–1136*
25. Sene M, Lee H, Diouf I, Toure M, Vonthron-Senecheau C, Auger C, Sarr M, Valerie Schini-KerthV, Kane MO. *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.*National Journal of Physiology, Pharmacy and Pharmacology*, 2020; Vol:10; Issue:9.DOI: 10.5455/njppp.2020.10.12394201921012020