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Research Article

Vasoactive properties of hydro-methanol pod powder extract of *Acacia nilotica* in porcine coronary artery: Role of Nitric Oxide (NO)

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ABSTRACT

Background: *Acacia nilotica* is a plant used in traditional medicine in Senegal for treatment of high blood pressure. The aim of this study was to determine whether a hydro-methanolic pod powder extract of *Acacia nilotica* (MSAN01) can induce a relaxant effect in porcine coronary arteries and to elucidate the underlying mechanism.

Methods: Porcine coronary artery rings were suspended in organ chambers to record changes in isometric forces. Rings with intact endothelium were incubated with or without L-Nitro Arginine (L-NA, 300 µM) to block NO synthase; 1,12 bis[(2-methylquinolin-4-yl)amino]dodecane (UCL, 100 nM), an inhibitor of small-conductance calcium-activated potassium channels (SKCa); and Tram-34 (1 µM), an inhibitor of intermediate-conductance calcium-activated potassium channels (IKCa); or indomethacin (INDO, 10 µM), a cyclooxygenase inhibitor, before contraction with U46619 (1-60 nM), a thromboxane A2 analogue, and subsequent generation of a concentration-relaxation curve to hydro-methanolic pod powder extract of *Acacia nilotica* (MSAN01). In some experiments, the endothelium was removed before contraction with U46619 (1-60 nM) and concentration-relaxation with MSAN01. Bradykinin was used to verify the presence of functional endothelium.

Results: Exposure The hydro-ethanolic pod powder extract of *Acacia nilotica* induces a vasodilatory effect in porcine coronary arteries pre-contracted with U46619. This effect is endothelium-dependent and mediated by nitric oxide (NO).

Conclusion: It *Acacia nilotica* induces vascular relaxation, which may explain the beneficial effects of this plant in the treatment of high blood pressure in Africa.

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1. Introduction

Lifestyle factors such as the consumption of hypercaloric or overly salted foods, smoking, alcohol consumption, and stress contribute to the emergence of diseases such as obesity, diabetes, and arterial hypertension (Krzesinski et al., 2002; Lawes et al., 2001). Hypertension accounts for 13.3% to 50% of cardiovascular diseases in Africa (Walker et al., 2000; Seck et al., 2010). These epidemiological data indicate that hypertension has become a significant public health issue (Pessinaba et al., 2013; Baldé et al., 2006). In Africa, many populations rely on medicinal plants to meet their healthcare needs.

The purpose of our study concerns *Acacia nilotica (Mimosaceae*), a plant widely distributed globally, particularly in sub-Saharan Africa, where it is traditionally used to treat various pathologies, including hypertension (Sene et al., 2023). However, these uses have not been conclusively validated by experimental scientific research. Therefore, the objective of our study is to determine the antihypertensive effects of Acacia nilotica by investigating its vasoactive properties on porcine coronary artery rings. Additionally, we aim to characterize the mechanisms underlying these effects.

2. Materials and methods

2.1 Plant Material

Pods of *Acacia nilotica* from the Fabaceae family were collected in February 2022 at the botanical garden of the Faculty of Medicine, Pharmacy, and Odontology at the Cheikh Anta Diop University of Dakar (Senegal). The pods 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. IFAN51885. The pods 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.

50 g grams of *Acacia nilotica* pods powder were subjected to decoction in 400 ml of methanol and 100 ml of boiling water for 5 min. After cooling, the decoction was filtered and the residue exhausted twice with the same volumes of methanol and water at each pass. The combined filtrate was then concentrated in a rotavapor and dried in an oven at 40°C. The hydro-methanolic extract obtained was then ground to produce a homogeneous dry powder (MSAN01) used for vascular reactivity experiments.

2.2 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.).

2.3 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).

The experimental protocols were conducted following the guidelines of the Institutional Ethics Committee (Research Ethics Committee of Cheikh Anta DIOP University).

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 A2 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 *Acacia nilotica* pods powder extract (MSAN01) in a cumulative manner to construct a relaxation-concentration curve.

2.4 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.

3. Results:

3.1 Role of endothelium in the vascular effects of MSAN01: MSAN01 induced relaxation of pig coronary artery rings having a functional endothelium and pre-contracted U46619 (Figure 1). The endothelium-dependent relaxations started at concentrations greater than 3 µg/ml and reached a maximal value close to 100 µg/ml (E_{max} = 99,56 %).

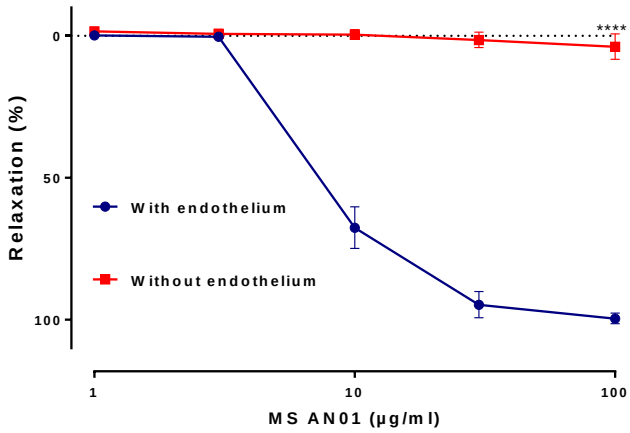

Fig.1. Effect-concentration curves of MSAN01 in isolated porcine coronary artery pre-contracted with U46619, with or without functional endothelium. Results are shown as means ± SEM of 6 different experiments. **** $p < 0.0001$ for inhibitory effect versus control.

3.2.Role of nitric oxide in the vasorelaxant effects of MSAN01: 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 MSAN01 (Figure 2).

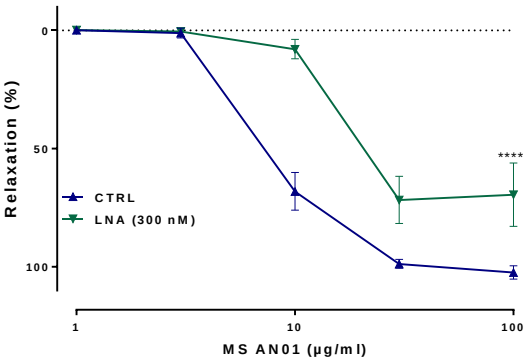

Fig.2. Effect-concentration curves of MSAN01 in isolated porcine coronary artery pre-incubated with L-NA (300 µM) for 30 minutes' incubation before addition of U46619. Results are shown as means ± SEM of 6 different experiments. **** $p < 0.0001$ for inhibitory effect versus control.

3.3.Role of prostacyclin in the vasorelaxant effects of MSAN01: 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 MSAN01 (Figure 3).

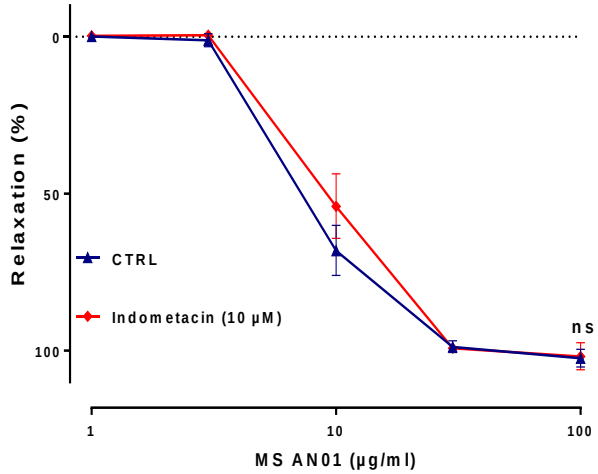

Fig. 3. Effect-concentration curves of MSAN01 in isolated porcine coronary artery pre-incubated with indomethacin (10 µM), for 30 minutes' incubation before addition of U46619. Results are shown as means ± SEM of 6 different experiments. n= 6

3.4. Role of* endothelium-derived hyperpolarizing factor *(EDHF) in the vasorelaxant effects of MSAN01: 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 MSAN01 (Figure 4).

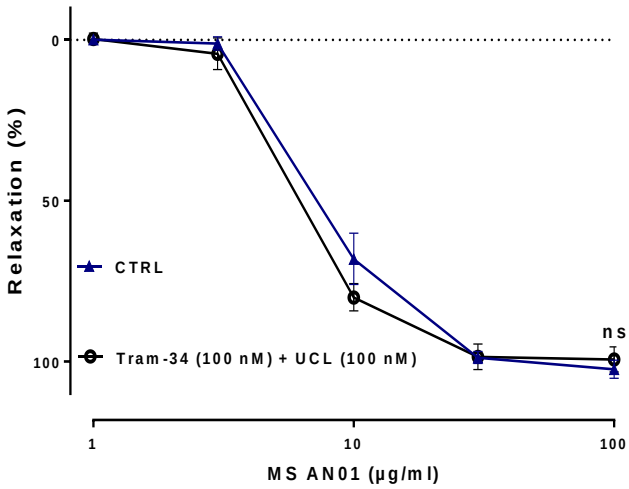

Fig. 4. Effect-concentration curves of MSAN01 in isolated porcine coronary artery pre-incubated with UCL (100 nM) + TRAM (100 nM) for 30 minutes' incubation before addition of U46619. Results are shown as means ± SEM of 6 different experiments. n= 6

4. Discussion

The results of this study demonstrate the in-vitro vasorelaxant effects of the hydroethanolic pod extract of *Acacia nilotica* (MSAN01) on porcine coronary arteries. Furthermore, the study elucidates the cellular and molecular signaling pathways involved in the vasorelaxation induced by this extract.

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: 99.56% 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 (Furchgott et al., 1983; Stoclet et al., 1999; Wallerath et al., 2002; Sene et al., 2019; Sene et al., 2020). 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 (PGI2) (Vanhoutte et al., 1999, 2001; Furchgott et al., 1980; Gauthier et al., 2005). The inhibition of endothelial NO synthase by L-NAME, resulting in a significant attenuation of vasorelaxation, highlights the central role of NO in the vasorelaxation induced by MSAN01. 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 (EDH) in the relaxation induced by MSAN01, reinforcing the notion of EDH's limited role in the vasorelaxation of large arterial trunks such as coronary arteries, as demonstrated by previous studies (De Moura et al., 2004; Kane et al., 2009). Another in-vivo study in rats showed antihypertensive properties of the metanolic fraction of the *Acacia nilotica* pod (Gilani et al., 1999). EDH is primarily active in microvessels, such as those in the mesenteric bed (Dal-Ros et al., 2009). 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 MSAN01 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 (Diallo et al., 2007; Fitzpatrick et al., 2000).

Relaxation mechanisms of vascular smooth muscle mediated by NO have been extensively described in the literature (Kane et al., 2009; Moncada et al., 1988; Rapoport et al., 1988). 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 (Rapoport et al., 1988).

Phytochemical analysis of MSAN01 has demonstrated that it is rich in polyphenols, including flavonoids, tannins, sterols, and triterpenes (Sene et al., 2023). 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 (Ndiaye et al., 2004). Our findings are consistent with these mechanisms. Collectively, our results suggest that the crude extract of *Acacia nilotica* induces vasorelaxation through the redox-sensitive activation of endothelial eNOS. Additional studies are required to confirm the results. Endothelial cell culture will be performed to induce phosphorylation of the endothelial NO synthase at serine 1177 and AKT at serine 473.

5. Conclusion

Acacia nilotica 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.

REFERENCES

Balde, M.D., Balde, N.M., Kaba, M.L. (2006). (2006). Hypertension artérielle : épidémiologie et anomalies métaboliques au Foutah-Djallon en Guinée. *Mali Med ; 3: 19-21*

Dal-Ros, C., Bronner, C., Schott, Kane, M.O., Chataigneau M., Schini-Kerth, V.B., Chataigneau, T. (2009). 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; 328:478–486*

De Moura, R.S., Miranda, D.Z., Pinto, A.C., Sicca, R.F., Souza, M.A., Rubenich, L.M., Carvalho, L.C., Rangel, B.M., Tano, T., Madeira, S.V., Resende, A.C. (2004). Mechanism of the endothelium dependent vasodilation and the antihypertensive effect of Brazilian red wine. *J Cardiovasc Pharmacol; 44(3):302-9.*

Diallo, A.S., Sarr, M., Mostafai, A., Pricci, M., Andrahtsitohaina, R. (2007). Cognac Polyphenolic Compounds increase Bradykinin-induced Nitric Oxide production in endothelial cells. *PhysioLResearch, inpress*

Fitzpatrick, D.F., Fleming, R.C., Bing, B., Maggi, D.A., O'Mailey, R.M. (2000). Isolation andcharacterization of endothelium-dependent vasorelaxing compounds from grape seeds. *J Agric Food Chem; 48:6384-90*

Furchgott, R.F. et al. (1983). Role of endothelium in reponses of vascular smooth muscle. *Circ Res; 53:557-73.*

Furchgott, R.F., Zawadzki, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature; 288: 373-6.*

Gauthier, K.M., Edwards, E.M., Falck, J.R., Reddy, D.S., Campbell, W.B. (2005). 14,15 epoxyeicosatrienoic acid represents a transferable endothelium-dependent relaxing factor in bovine coronary arteries. *HTA; 45: 666- 71.*

Gilani, A.H., Shaheen, F., Zaman, M., Janbaz, K.H., Shah, B.H., Akhtar, M.S. (1999). Studies on antihypertensive and antispasmodic activities of methanol extract of Acacia nilotica pods. *Phytother Res*. ;13(8):665-9. doi: 10.1002/(sici)1099-1573(199912)13:8<665::aid-ptr563>3.0.co;2-t. PMID: 10594935.

Kane, M.O., Anselm, E., Rattmann, Y.D., Auger, C., Schini-Kerth, V.B. (2009). Role of gender and estrogen receptors in the rat aorta endothelium-dependent relaxation to red wine polyphenols. *Vascul Pharmacol; 51(2-3): 140-6.*

Krzesinski, J.M. (2002). Épidémiologie de l'hypertension artérielle. *Rev Med Liège; 57(3): 142-7*

Lawes, C., Vander, H.S., Rodgers A. (2001). Global burden of blood-pressure-related disease. *Lancet; 371:1513-1518.*

Moncada, S., Radonski, M.W., Palmer, R.M.J. (1988). Endothelium-derived relaxing factor, identification as NO and role in the control of vascular tone and platelet function. *Biochem. Pharmacol; 37:2495-501*

Ndiaye, M., Chataigneau, T., Chataigneau, M., Schini-Kerth, V.B. (2004). 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 ; 142 : 1131–1136*

Ndiaye, M., Chataigneau, M., Lobysheva, I., Chataigneau, T., Schini-Kerth, V.B. (2005). Red wine polyphenol-induced, endothelium-dependent NO-mediated relaxation is due to the redox-sensitive PI3-kinase/Akt-dependent phosphorylation of endothelial NO-synthase in the isolated porcine coronary artery. *FASEB J; 19: 455–457.*

Pessinaba, S., Mbaye, A., Yabeta, G.A.D., Ndao, C.T., Harouna, H., Diagne, D., Diack, B., Kane, M., Kane, A., Ndiaye, M.B., Bodian, M., Diao, M., Mbaye, M.N., Niang, K., Sy, J.B. (2013). Prevalence and determinants of hypertension and

associated cardiovascular risk factors: data from a population based, cross-sectional survey in Saint Louis, Senegal. *Cardiovasc J Afr. ;24(5):180-183.*

Rapoport, R.M., Diaznin, M.B., Murad, F. (1983).Endothelium-dependent relaxation in rat aorta may be mediated through cyclic GMP-dependent protein phosphorylation. *Circ Res.;52(3):352-7.*

Seck, S.M., Guéye, S., Tamba, K., Ba I. (2013). Prevalence of Chronic Cardiovascular and Metabolic Diseases in Senegalese Workers: A Cross-Sectional Study, 2010. *Prev Chronic Dis; 10:110339. doi:10.5888/pcd10.110339.*

Sene, et al (2023). Healing, Anti-inflammatory and Analgesic Activities of the Hydro-Methanolic Extract of Acacia nilotica Pods (Mimosaceae), *Int. J. Pharmacol.*, vol. 19, n° 1, p. 40-51, doi: 10.3923/ijp.2023.40.51.

Sene, M., Diouf, I., Toure, M., Auger, C., Senecheau, C.V., Sarr, M., Schini-Kerth, V., Kane, M.O. (2019). Vasoactive properties of Ceiba pentandra in porcine coronary artery and different conductance and resistance vessels from rats: A role of nitric oxide. Natl J Physiol Pharm Pharmacol ;9(10):1027-1033.

Sene, M., Lee, H., Diouf, I., Toure, M., Senecheau, C.V., Auger, C., Sarr, M., Schini-Kerth, V., Kane, M.O. (2020) *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. *Natl J Physiol Pharm Pharmacol* ;10(09):804-812.

Stoclet, J.C., Kleschyov, A., Andriambeloson, E., Diebolt, M., Andriantsitohaina, R. (1999). Endothelial no release caused by red wine polyphenols. *J Physiol Pharmacol; 50, 535-540.*

Walker, R.W., McLarty, D.G. (2000). Kitange HM. Stroke mortality in urban and rural Tanzania. *Lancet, 355: 1684-7.*

Wallerath, T., Deckert, G., Ternes, T., Anderson, H., Li, H., Witte, K., Forstermann, U. (2002). Resveratrol, a polyphenolic phytoalexin present in red wine, enhances expression and activity of endothelial nitric oxide synthase. *Circ.; 106, 1652-1658.*

Vanhoutte, P.M. (2001). Endothelium-derived free radicals: for worse and for better. *J Clin Invest; 707, 23-25.*

Vanhoutte, P.M. (1999). How to assess endothelial function in human blood vessels. *J Hypertens; 17: 1047-58.*