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Endothelium dependent and endothelium independent activity of ethanolic extract of *Moringa oleifera* Lam. (Moringaceae) on porcine coronary arteries and its underlying mechanisms of vasorelaxation

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Abstract
The results showed that ethanolic and aqueous extract induce relaxation in endothelium intact porcine coronary artery rings precontracted with U46619, with better activity of ethanolic extract. In endothelium-denuded coronary artery, the relaxation effects of ethanolic extract were slightly but significantly affected. In endothelium intact coronary artery, the presence of N^{ω}-nitro-L-arginine (NO-synthase inhibitor) or Wortmannin (PI3-kinase inhibitor) were not avoid the vasorelaxant effect of ethanolic extract, but were significantly shifted compared to the control. Moreover, the presence of the membrane-permeant analogue of SOD (MnTMPyP) or cyclooxygenase inhibitor (indomethacin) has not prevented the relaxation effect of the extract. In addition, in the KCl (60mM) induced vasoconstriction in endothelium denuded coronary arteries, ethanolic extract produced a maximum effect of 47.35% compared to the vehicle. Taking together, these findings indicate that *Moringa oleifera* induce an endothelium dependent and endothelium independent vasorelaxation effect in porcine coronary artery which may involve a voltage dependent calcium pathways.

Keywords: *Moringa oleifera*, coronary arteries, vasorelaxant, nitric oxide, NO-synthase, PI3-Kinase/Akt

1. Introduction
Arterial hypertension (HT) constitutes an important risk factor for developing other diseases as endothelial dysfunction, metabolic syndrome, diabetes, renal dysfunction, congestive heart failure, coronary artery disease and stroke, due to the deleterious effects it exerts on the vascular systems of these organs [1, 2]. Abnormal vascular reactivity, including impaired endothelium dependent relaxation and enhanced sensitivity to vasoconstrictors, is a hallmark of hypertensive disease. Therefore, the relaxation of vascular smooth muscles is very crucial in hypertension and other cardiovascular diseases [3]. Relaxation of vascular smooth muscles is one of the strategies for the treatment of hypertension [4]. A considerable number of bioactive compounds derived from plant material have been shown to possess cardioprotective effect and reduce the risk of cardiovascular disease [5]. *Moringa oleifera* leaves contain several bioactive phytochemicals such as alkaloids, anthocyanin, anthraquinone, cardiac glycosides, carotenoids, flavonoids, saponins, steroids, tannins, quercetin, kæmpferia, etc. [6-9] known for their cardioprotective, vasorelaxant and antihypertensive effects [10, 11, 5]. This study aimed to characterize the vascular actions of ethanolic extract of *Moringa oleifera* (EEM) as well as to elucidate the underlying mechanisms of action in vasodilation in isolated porcine coronary arteries.

2. Material and methods
2.1 Plant material and extraction
The fresh leaves of *Moringa oleifera* were harvested in the southwest of Burkina Faso in July 2010. It was identified in the Bio-Info Center of University Ouaga1 Pr Joseph Ki-Zerbo where a voucher specimen n°16869 was deposited. The leaves were dried at room temperature and crushed into powder. The resulting powder was macerated and soaked at room temperature in 70 ° ethanol (10 % g / v) for 72 hours. After that, the macerate is filtered and concentrated in a rotary evaporator under reduced pressure. The residue obtained was dried. Yield 16,09%.

2.2 Reagents
Thromboxane A2 analogue, the 9,11-dideoxy-11a,9a-epoxy-methano-prostaglandin F2α, U46619 Cayman Chemical (Ann Arbor, MI, USA), indomethacin were purchased from Sigma Chemical Co. (Grenoble, France); N^{ω}-nitro-L-arginine (LNNA), wortmannin and Mn(III)

tetrakis (1-methyl-4-pyridyl) porphyrin pentachloride (MnTMPyP) were purchased at Enzo Life Sciences (Villeurbanne, France).

2.3 Preparation of porcine coronary artery rings

Porcine hearts were obtained from a local abattoir and kept refrigerated before transfer to the laboratory. The left anterior descending coronary artery was dissected free from the surrounding myocardium. After cleaning of adherent fat and connective tissue, the artery was cut into rings of 3–4 mm length, three (3) coronary arterial rings were prepared from each heart. The rings were suspended horizontally between two parallel stainless steel hooks for the measurement of isometric tension in individual organ bath containing Krebs solution (in mM): NaCl 119, KCl 4.7, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $NaHCO_3$ 25, $CaCl_2$ 1.25 and glucose 11, maintained at 37°C and continuously bubbled with 95% O_2 5% CO_2 mixtures. Tension was recorded using isometric transducer (type EMKA Technologies) and was connected to an analogical amplifier (ps100W-2; EMKA Technologies).The graphic recording was obtained by the software of acquisition U-vessel (WAGNER, 1999; France). Rings were stretched to 5 g of baseline tension and equilibrated for 90 min. During the equilibration time Krebs solution was replaced every 15 min in organ bath. After that, rings were pre-contracted with thromboxane A2 mimetic, U46619 (1.10^{-8} to 3.10^{-8} M). After equilibration period, arteries were challenged with KCl (80 mM) in order to test vessel viability. After each experience Krebs solution was replaced two times in organ bath before following test. The absence or presence of endothelial cells was confirmed by the absence or presence of relaxation to the endothelium-dependent vasodilator bradykinin (10^{-6} M). Relaxation response of endothelium intact porcine coronary arteries (EIPCA) or endothelium denuded arteries (EDPCA) rings to cumulative concentration of AEM or EEM (10^{-2} to 1 mg/ml) was performed after precontraction with U46619 (1.10^{-8} to 3.10^{-8}M). Endothelium removal was performed by gently rubbing with a wooden probe with cotton. To study the underling effect of the plant extract, some inhibitors were used. Indeed, EIPCA rings were pre-incubated during 30 min with non-specific nitric oxide synthase inhibitor, N^{ω}-nitro-L-arginine (LNNA, 300µM), or with an indomethacin (10 µM, non-specific cyclooxygenase (COX) inhibitor), or with Wortmannin (30 nM, PI3-kinase/Akt inhibitor), or with membrane permeant analogues of superoxide dismutase (SOD), MnTMPyP (100 µM) before precontraction with U46619. Relaxation response of coronary artery rings to cumulative concentrations of EEM (10^{-2} to 1 mg/ml) was then performed in the presence or absence of endothelium. Voltage dependent calcium channels involvement in EEM induced vasorelaxation was assessed using cumulative concentration dependent-vasorelaxant curve of EEM in KCl (60 mM) precontracted EDPCA rings.

2.4 Statistical analysis

All data were expressed as means ± standard error of the mean (S.E.M.). Statistical analysis was conducted using two way ANOVA followed by Dunnett post test. A value of $p<0.05$ was considered statistically significant. All analyses were performed with Graph Pad Prism 5.00 (Graph Pad software, San Diego, CA, USA).

3. Results

3.1 EEM induced vasodilation in porcine coronary artery rings precontracted with U46619

In this study, we already compare the effect of EEM and the aqueous extract of *Moringa oleifera* (AEM) that is the mode of traditional use of the plant.

In rings with endothelium, EEM and AEM at 10^{-2} to 1 mg/mL significantly relaxed the sustained contractions induced by U46619 in a concentration-dependent manner (Fig.1). The better relaxation was obtained with EEM (Emax = 103.33 ± 3.24%) compared to AEM (Emax = 46.73 ± 13.25%) with a significant difference. Then, we decided to investigate the effects of EEM in this study in order to underline its possible mechanism of relaxation effect.

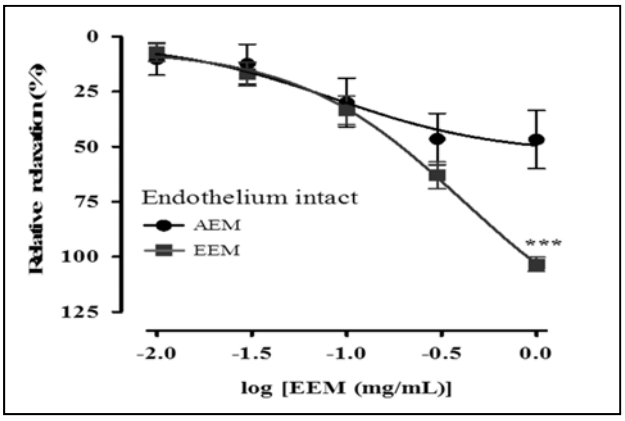

Fig 1: Relaxing effects of EEM on porcine coronary artery rings in the presence of endothelium. Relative relaxation were expressed as the percentage decreased in the contraction to U46619.Values are means ± S.E.M. (n = 8). ***$p<0.001$ compared to AEM.

3.2 EEM induced vasodilation effect in the presence and absence of endothelium in porcine coronary artery rings.

To investigate the effects of EEM on the coronary artery, rings with endothelium and without endothelium were used.

The results show that EEM induces vasorelaxation effects in EIPCA rings as well as in EDPCA rings (Fig.2). This relaxation in the presence of endothelium is significantly different from that in the absence of endothelium indicating an endothelium dependent and an endothelium independent vasodilation effect of EEM. The solvent showed no effect on the contraction induced by U46619.

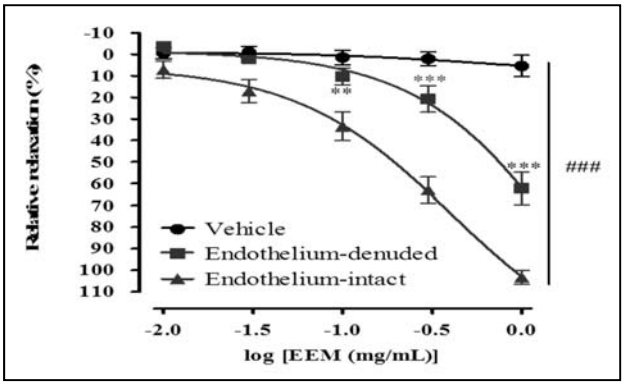

Fig 2: Effects of EEM-induced relaxations in endothelium intact and endothelium denuded porcine coronary artery rings (**$p<0.01$, ***$p<0.001$ endothelium-denuded versus endothelium-intact; ###$p<0,001$ endothelium-denuded and endothelium intact versus vehicle); n= 8-11.

3.3 Role of prostanoïds in the vasodilation effects of EEM

This test was investigated to examine the role of prostacyclin in the endothelium-dependent relaxation of EEM in the porcine coronary artery.

Endothelium intact rings were pre-incubated with indomethacin (10µM) before contraction with U46619, followed by cumulative concentrations of EEM. The results showed that indomethacin had no effect on the endothelium-dependent relaxations of EEM (Fig.3).

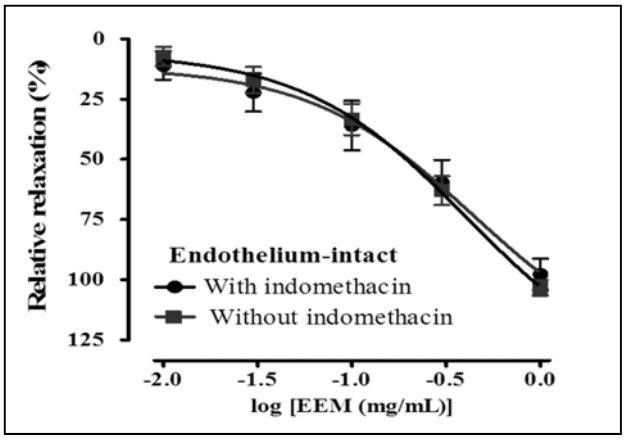

Fig 3: Vasorelaxant effects of EEM on endothelium intact porcine coronary arteries in the presence or absence of indomethacin (10 µM); (*p<0.05, n =7).

3.4 Role of nitric oxide pathway in the relaxation effect of EEM in porcine coronary artery rings

To evaluate the involvement of nitric oxide in the EEM-induced vasorelaxation, rings with endothelium were preincubated with NOS-inhibitor, Nω-nitro-L-arginine, (L-NNA, 300 µM) before the cumulative concentration of EEM (Fig.4). EEM (0.01– 1mg/ml) induced relaxation in a concentration-dependent manner both in endothelium-intact and -denuded porcine coronary artery rings precontracted with U46619 (Fig. 4). The relaxant effect of EEM in EIPCA and EDPCA rings was significantly different with a better effect in the presence of endothelium. The EC_{50} values were 0.41 mg/ml and 2.31mg/ml, respectively in the presence and absence of endothelium. Similarly, there were a significant differences between the Emax values of EEM in intact (Emax = 103.327 ± 3.243%, EC_{50} = 0.41mg/ml) and denuded (Emax = 62.302 ± 7.46%, EC_{50} = 2.31mg/ml) porcine coronary rings pre-contracted with U46619.

Likewise, a significant difference (p<0.05) was found in the pD_2 value of EEM in intact and denuded rings (0.39 and 0.36 respectively). Thus, endothelium removal reduced significantly but not blocked relaxant effect of EEM.

However, in the presence of L-NNA, the results showed that the vehicle alone had no striking relaxation effect on artery rings.

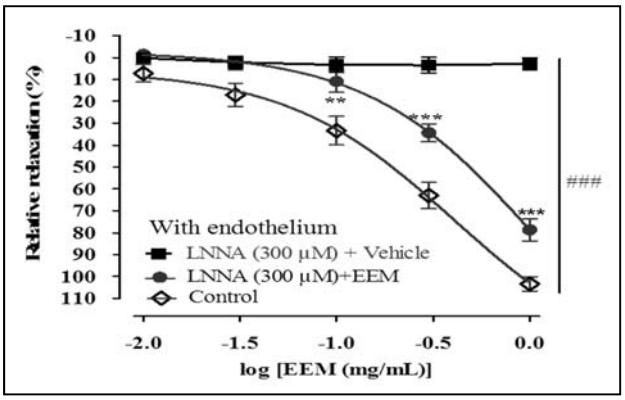

Fig 4: Effect of EEM on U46619-induced contractile response in endothelium intact porcine coronary artery rings preincubated with L-NNA (300µM). The curves were determined in the presence of vehicle (■) or an increase concentration of EEM (0.01 to 1 mg/mL). (**p<0.01, ***p<0.001, versus control; ###p<0.001, control and LNNA+EEM versus LNNA+ vehicle; n= 6-11).

3.5 Relationship between intracellular reactive oxygen species and PI3-Kinase/Akt inhibition on EEM vasodilation effect in porcine coronary arteries

The involvement of PI3-kinase/Akt and ROS pathways were explored in endothelium-intact porcine coronary artery rings (Fig. 5). The results showed that vasorelaxant effects of EEM (Emax = 103.33 ± 3.24%; EC_{50} = 0.41 mg/ml) was significantly inhibited in the presence of Wortmannin (Emax = 85.18 ± 5.89% and EC_{50} = 0.61mg/ml), a PI3 kinase/Akt inhibitor.

Moreover, the role of intracellular reactive oxygen species in the endothelium-dependent relaxation to EEM was also assessed (Fig.6). In porcine coronary artery preincubated with MnTMPyP (membrane-permeant analogue of superoxide dismutase), results showed no potency effect on vasorelaxant effects of EEM (●, control). Nevertheless, a little significant effect was observed at 0.1 mg/mL with no difference between their maximum effect (Emax = 106.01 ± 8.36%). Moreover, no significant difference was obtained between their half maximal effective concentration (EC_{50} = 0.41mg/ml and 0.79mg/ml in the control and in the presence of MnTMPyP respectively).

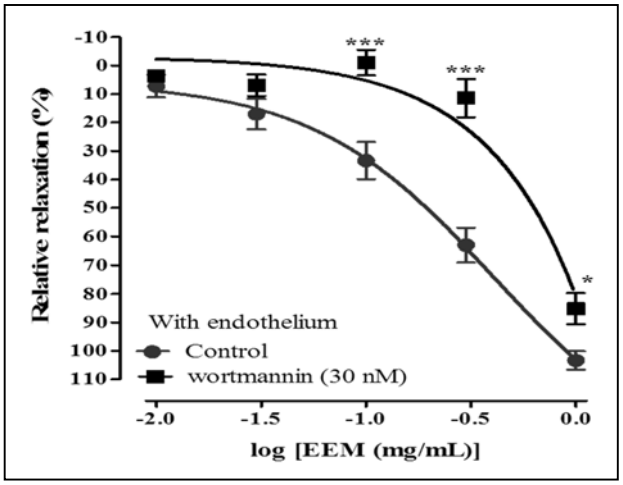

Fig 5: Effect of EEM on U46619-induced contractile response in EIPCA rings preincubated with wortmannin (30 nM). (*p<0.05, ***p<0.001, versus control, n = 11).

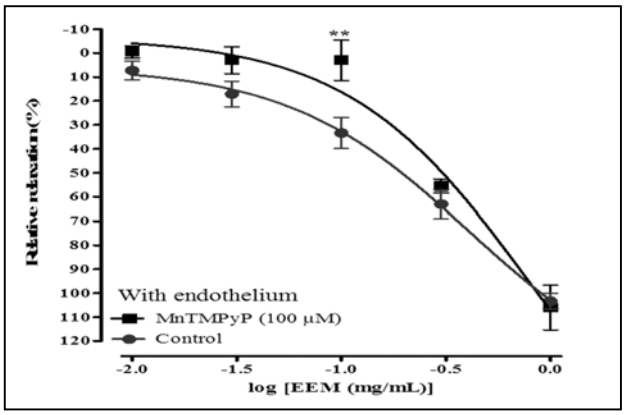

Fig 6: Effect of EEM on U46619-induced contractile response in endothelium intact porcine coronary artery rings preincubated with MnTMPyP (100 µM). The curves were determined by adding increased concentration of EEM (0.01-1 mg/mL). (**p<0.01, n = 5-11).

3.6 Effects of EEM on high K^+ induced vasoconstriction in porcine coronary arteries.

This experiment was done to verify the effect of EEM in KCl-induced contractile response in porcine coronary artery rings. KCl (60 mM) was used to induce contraction in EDPCA rings and then EEM was added cumulatively (0.01 – 1mg/mL). The EEM-induced concentration-dependent relaxation was calculated as a percentage of the relaxation in response to KCl.

The results showed that EEM induces vasodilation effect on the vasoconstriction induced by KCl in a concentration dependent manner (Emax = 47.35 ± 5.69 %) compared to the vehicle that remain contracted in the absence of EEM (Fig. 7).

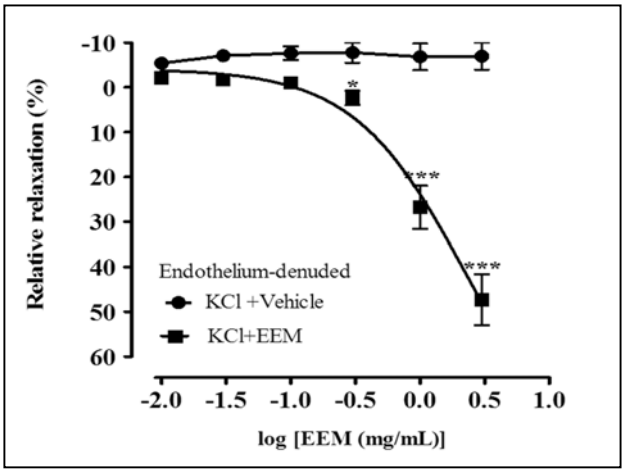

Fig 7: Vasorelaxant effects of EEM on vasoconstriction induced by KCl (60 mM) in endothelium denuded porcine coronary arteries rings (*p<0.05; ***p<0.001; n = 5).

4. Discussion

The present study aims to underline the mechanism of action of *Moringa oleifera* plant extracts on vascular system.

Administration of ethanolic extract (EEM) or aqueous extract (AEM) of *Moringa oleifera* entirely reversed the stimulated vasoconstriction induced by 9,11-dideoxy-11a,9a-epoxymethanoprostaglandin F2a (U46619), a thromboxane sympathomimetic (fig.1).

Indeed, thromboxane prostanoid (TP) receptors cause potent vasoconstriction, which contributes to increase vascular tone and blood pressure. TP receptor activation attenuates endothelium-dependent relaxations in response to the β-adrenoceptor agonist *via* RhoA/Rho kinase mediated but calcium-independent mechanisms, leading to the reduced eNOS activation and NO production in endothelial cells [12]. In the present study, EEM showed more important (Emax = 103.327 ± 3.243%) vasorelaxant effects than AEM (Emax = 46. 73%) on EIPCA rings. Then, we decided to investigate the effects of EEM in this study in order to underline its possible mechanism of relaxation effect. This result is comparable to those of Branković *et al*. (2010) on *Apium graveolens* [13]. Arend *et al*. (2015) showed that the amount of phenolic compounds in the ethanolic extracts was higher than that of the aqueous extracts of *Cecropia glaziovii* [14]. Indeed, polyphenolic compounds are known for their vasorelaxant and cardioprotective effects [15, 6].

Leaf extracts of *M. oleifera* are reported to be rich in compounds such as crypto- chlorogenic, isoquercetin, astragalin, myricetin, quercetin, kaempferol and other flavonoids known for their antioxidant, anti-radical scavenging, vasorelaxant, anti-inflammatory, antihypertensive and cardioprotective activities [16, 17, 6, 9]. The presence of polyphenolic compounds such as flavonoids, quercetin, kaempferol in *Moringa oleifera* leaves extract has been widely reported by many authors [6, 9] showed that flavonoid inhibited cyclic nucleotide phosphodiesterase type 1 (PDE1) and caused cGMP-dependent protein kinase (PKG) vasorelaxant in human vascular tissue [15]. Torres-Piedra *et al*. (2011) showed that vasorelaxant effect of flavonoids could be mediated through calmodulin inhibition [18]. The protein calmodulin (CaM), is a major cellular Ca^{2+}-binding protein which regulates the activity of a series of CaM-dependent enzymes such as NO synthases, phosphodiesterases (PDE's), adenylate cyclases, phosphatases, several kinases, ion channels, calcium-ATPase pumps, among others. Mansour *et al*. (2014) showed that *Moringa* leaf extract induces significant recovery in cholesterol, triglyceride and LDL-C levels in gamma-irradiation induced significant increase in serum levels of cholesterol, triglycerides and LDL-C in rat [19]. Many authors associate more often the decrease in cholesterol level and inhibition of the main enzyme of the synthesis of cholesterol, 3-hydroxy-3-methylglutaryl-CoenzymeA (HGM-CoA) [20-22]. In vascular endothelial cells, this inhibition of HMG-CoA reductase leads to upregulation of the expression and activity of endothelial nitric oxide synthase [23, 24] and then leads to NO-dependent vasorelaxation.

The vasorelaxant effects of EEM in EIPCA and EDPCA rings were investigated. The results showed that EEM induces vasorelaxation in EIPCA rings as well as in EDPCA rings (Fig.2). This relaxation in the presence of endothelium was significantly affected by removal of endothelium indicating an endothelium dependent and an endothelium independent vasodilation effect of EEM. Preincubation with indomethacin, a cyclo-oxygenase (COX) inhibitor, did not affected the vasorelaxation induced by EEM in EIPCA rings (Fig.3), suggesting that the endothelium-dependent-relaxation effect of EEM is not mediated by PGI2, but probably by another mechanism which remains to be investigated.

On the other hand, NOS-inhibitor, Nω-nitro-L-arginine, (L-NNA, 300 µM), significantly, inhibited the EEM–induced relaxation in EIPCA rings (Fig.4). This finding suggests an involvement of the eNOS-NO-cGMP pathway in the endothelium-dependent vasorelaxation of EEM on porcine

coronary arteries. Indeed, guanylyl-cyclase (GC) catalyzes the conversion of GTP to cyclic GMP (cGMP), which in turn activates the cGMP-dependent protein kinase (PKG). The activated PKG then phosphorylates several important target proteins including ion channels, ion pumps, receptors, and enzymes, leading to a decrease in intracellular Ca^{2+} and relaxation of the smooth muscle cells [25, 26].

The involvement of PI3-kinase/Akt and ROS pathways were explored in endothelium-intact porcine coronary artery rings. They constitute the upstream of NO-synthase activation process and subsequent release of NO in endothelial cells. The results showed that vasorelaxant effect of EEM in EIPCA rings was significantly inhibited in the presence of Wortmannin, a PI3-kinase/Akt inhibitor (Fig.5). These findings suggest that EEM-induces vasorelaxation *via* activation of Akt-eNOS-cGMP signaling [27].

Also, membrane-permeant analogue of SOD, MnTMPyP, induced a small but significant difference with the control (Fig.6). No difference was observed between their maximum effects. These results are comparable to those of Belemnaba *et al*. (2013); Tokoudagba *et al*. (2010) on porcine coronary arteries [28, 29]. According to Kim *et al*. (2013), in healthy arteries, polyphenols and polyphenol-rich sources could induce a formation of ROS specifically in the endothelial cells, leading to the activation of the Src/PI3-kinase/Akt pathway and eNOS with the subsequent formation of NO and vasorelaxation [30]. Stoclet *et al*. (2004) reported that ROS, especially superoxide anions, act as upstream mediators of Src kinase whereas both superoxide anions and hydrogen peroxide are involved in the activation of the PI3-kinase/ Akt pathway leading to eNOS phosphorylation [31].

To explore endothelium independent vasorelaxation mechanism of EEM, voltage-dependent calcium channels pathways was assessed. Thus, EEM relaxed vasoconstriction induced by KCl in EDPCA rings through the activation of voltage-dependent calcium channels (Fig7). This result suggests that EEM induces its action by blocking the voltage-dependent calcium channel. Contractile response in smooth muscle is caused by an influx of Ca^{2+} through voltage-dependent Ca^{2+}-channels and/or receptor-operated Ca^{2+}-channels [32]. This result with KCl is comparable to those of Dimo *et al*. (2007) [33].

The present study indicates that EEM is a strong vasodilator of porcine coronary arteries. These preliminary results suggest that the mechanism of relaxation seems to be mediated by stimulating the endothelial formation of NO. They also indicate that the intracellular formation of ROS in endothelial cells (in particular superoxide anions and hydrogen peroxide) leading to activation of eNOS *via* the Src/PI3-kinase/Akt pathway is involved. Vasorelaxant effect of EEM may also be attributed to inhibitory effects on voltage-dependent calcium channels and/or receptor-operate calcium channels; however, other mechanisms could not be ruled out. In future investigations, we will explore the effect of EEM on the receptor-operated calcium channels and other receptors operated ions channels in order to clarify the endothelium independent mechanisms of the extract. The present results provide pharmacological support for the use of *Moringa oleifera* in ethnomedical practices as vasorelaxant and antihypertensive in Burkina Faso.

5. References

1. Vergara-Galicia J, Ortiz-Andrade R, Rivera-Leyva J, Castillo-España P, Villalobos-Molina R, Ibarra-Barajas M *et al*. Vasorelaxant and antihypertensive effects of methanolic extract from roots of *Laelia anceps* are mediated by calcium-channel antagonism. Fitoterapia, 2010; 81:350-357.
2. González-Quevedo A, González-García S, Hernández-Díaz Z, Concepción OF, Sotolongo LQ, Peña-Sánchez M *et al*. Serum neuron specific enolase could predict subclinical brain damage and the subsequent occurrence of brain related vascular events during follow up in essential hypertension. Journal of the Neurological Sciences. 2016; 363:158-163
3. Bankar GR, Nandakumar K, Nayak PG, Thakur A, Chamallamudi MR, Nampurath GK. Vasorelaxant effect in rat aortic rings through calcium channel blockage: A preliminary in vitro assessment of a 1, 3,4-oxadiazole derivative. Chemico-Biological Interactions. 2009; 181:377-382.
4. Awadallah FM, El-Eraky WI, Saleh DO. Synthesis, vasorelaxant activity, and molecular modeling study of some new phthalazine derivatives. European Journal of Medicinal Chemistry. 2012; 52:14-21.
5. Mondolis E, Morán-Pinzón JA, Rojas-Marquéz FA, López-Pérez JL, Abad A, Amaro-Luis JM *et al*. Vasorelaxant effects in aortic rings of eight diterpenoids isolated from three Venezuelan plants. Rev Bras Farmacogn. 2013; 23:769-775.
6. Vongsak B, Sithisarn P, Gritsanapan W. Bioactive contents and free radical scavenging activity of *Moringa oleifera* leaf extract under different storage conditions. Industrial Crops and Products. 2013; 49:419-421.
7. Nweze NO, Nwafor FI. Phytochemical, proximate and mineral composition of leaf extracts of Moringa oleifera Lam. from Nsukka, South-Eastern Nigeria. IOSR. Journal of Pharmacy and Biological Sciences. 2014; 9:99e103.
8. Vanajakshi V, Vijayendra SVN, Varadaraj MC, Venkateswaran G, Agrawal R. Optimization of a probiotic beverage based on Moringa leaves and beetroot. LWT - Food Science and Technology, 2015; 63:1268-1273.
9. Matshediso PG, Cukrowska E, Chimuka L. Development of pressurised hot water extraction (PHWE) for essential compounds from Moringa oleifera leaf extracts. Food Chemistry. 2015; 172:423-427.
10. Ndiaye M, Chataigneau T, Andriantsitohaina R, Stoclet JC, Schini-Kerth VB. Red wine polyphenols cause endothelium-dependent EDHF-mediated relaxations in porcine coronary arteries via a redox-sensitive mechanism. Biochemical and Biophysical Research Communications. 2003; 310:371-377
11. Perez-Vizcaino F, Duarte J, Jimenez R, Santos-Buelga C, Osuna A. (Antihypertensive effects of the flavonoid quercetin. Pharmacol Rep., 2008, 61:67-75.
12. Liu CQ, Leung FP, Wong SL, Wong WT, Lau CW, Lu L *et al*. Thromboxane prostanoid receptor activation impairs endothelial nitric oxide-dependent vasorelaxations: The role of Rho kinase; Biochemical Pharmacology. 2009; 78:374-381.
13. Branković S, Kitić D, Radenković M, Veljković S, Kostić M, Miladinović B *et al*. Hypotensive and cardioinhibotory effects of the aqueous and ethanolic extracts of Celery (*Apium graveolens*, APIACEAE). Acta Medica Medianae, 2010; 49(1):13-16.
14. Arend DP, dos Santos TC, Cazarolli LH, Hort MA, Sonaglio D, dos Santos ALG *et al*. *In vivo* potential

hypoglycemic and in vitro vasorelaxant effects of Cecropia glaziovii standardized extracts. Revista Brasileira de Farmacognosia. 2015; 25:473-484.
15. Gonçalves RL, Lugnier C, Keravis T, Lopes MJ, Fantini FA, Schmitt M *et al*. The flavonoid dioclein is a selective inhibitor of cyclic nucleotide phosphodiesterase type1 (PDE1) and a cGMP-dependent protein kinase (PKG) vasorelaxant in human vascular tissue. European Journal of Pharmacology. 2009; 620:78-83.
16. Duarte J, Jimenez R, Villar IC, Perez-Vizcaino F, Jimenez J, Tamargo J. Vasorelaxant effects of the bioflavonoid chrysin in isolated rat aorta. Planta Med. 2001; 67:567-569.
17. Penso J, Cordeiro KCFA, da Cunha CRM, Castro PFDS, Martins DR, Lião LM *et al*. Vasorelaxant activity of 7-β-O-glycosides biosynthesized from flavonoids. European Journal of Pharmacology. 2014; 733:75-80.
18. Torres-Piedra M, Figueroa M, Hernández-Abreu O, Ibarra-Barajas M, Navarrete-Vázquez G, Estrada-Soto S. Vasorelaxant effect of flavonoids through calmodulin inhibition: Ex vivo, in vitro, and in silico approaches. Bioorganic & Medicinal Chemistry. 2011; 19:542-546
19. Mansour HH, Ismael NER, Hafez HF. Modulatory effect of Moringa oleifera against gamma-radiation-induced oxidative stress in rats. Biomedicine & Aging Pathology. 2014; 4:265-272.
20. Belkheiri N. Dérivés phénoliques à activités antiatherogènes. Doctorat de l'Université de Toulouse. 2010, 18-26.
21. Bouguerne B. Conception et synthèse des dérivés phénoliques hautement fonctionnalisés et étude de leurs propriétés biologiques vis-à-vis des maladies cardiovasculaires (athérosclérose). Doctorat de l'Université de Toulouse. 2012, 40-43.
22. Oumaroua BFA, Ndzanaa MTB, Ngo Lemba ET, Bilanda DC, Dimo T. Antihypertensive Activity Of Jateorhiza Macrantha (Menispermaceae) Aqueous Extract On Ethanol-Induced Hypertension In Wistar. Int J Pharm Pharm Sci. 2012; 4(2):293-298.
23. Laufs U, La Fata V, Plutzky J, Liao JK. Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors. Circulation. 1998, 1129-1135.
24. Döndaş NY, Sucu N, Yilmaz BC, Kaplan HM, Özeren M, Karaca MK *et al*.. Molecular mechanism of vasorelaxant and antiatherogenic effects of the statins in the human saphenous vein graft. European Journal of Pharmacology 2011; 666:150-157.
25. Friebe A, Koesling D. Regulation of nitric oxide-sensitive guanylyl cyclase. Circulation Research. 2003; 93:96-105.
26. Dongmo AB, Azebaze AGB, Donfack FM, Dimo T, Nkeng-Efouet PA, Devkota KP *et al*. Pentacyclic triterpenoids and ceramide mediate the vasorelaxant activity of Vitex cienkowskii via involvement of NO/cGMP pathway in isolated rat aortic rings. Journal of Ethnopharmacology. 2011; 133:204-212.
27. Chataigneau T, Ndiaye M, Schini-Kerth VB. Effets vasodilatateurs des composés polyphénoliques du vin : rôle de NO et EDHF. Sang Thrombose Vaisseaux 2003; 15(8):433-41.
28. Belemnaba L. Propriétés antihypertensive de Anogeissus leiocarpus (DC) Guill. et Perr. (Combretaceae) et Lannea microcarpa Engl. et K. Krausse (Anacardiaceae), plantes médicinales du Burkina Faso: Etude *in vivo* de leurs mécanismes d'action au niveau vasculaire. Thèse Univ. Ouaga. 2012, 96.
29. Tokoudagba JM, Auger C, Bréant L, N'Gom S, Chabert P, Idris-Khodja N *et al*. Procyanidin-rich fractions from Parkia biglobosa (Mimosaceae) leaves cause redox-sensitive endothelium-dependent relaxation involving NO and EDHF in porcine coronary artery. Journal of Ethnopharmacology. 2010; 132:246-250.
30. Kim JH, Auger C, Kurita I, Anselm E, Rivoarilala LO, Lee HJ *et al*. Aronia melanocarpa juice, a rich source of polyphenols, induces endothelium-dependent relaxations in porcine coronary arteries via the redox-sensitive activation of endothelial nitric oxide synthase. Nitric Oxide. 2013; 35:54-64
31. Stoclet JC, Chataigneau T, Ndiaye M, Oak MH, Bedoui JE, Chataigneau M *et al*. Vascular protection by dietary polyphenols. European Journal of Pharmacology. 2004; 500:299-313.
32. Matsuo H, Okamoto R, Zaima K, Hirasawa Y, Ismail IS, Lajis NH *et al*. New vasorelaxant indole alkaloids, villocarines A–D from Uncaria villosa. Bioorganic & Medicinal Chemistry. 2011; 19:4075-4079.
33. Dimo T, Mtopi OSB, Nguelefack TB, Kamtchouing P, Zapfack L, Asongalem EA *et al*. Vasorelaxant effects of Brillantaisia nitens Lindau (Acanthaceae) extracts on isolated rat vascular smooth muscle. J of Ethnopharmacol. 2007; 111:104-109.