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Vasodilator effect of *Zanthoxylum zanthoxyloïdes, Calotropis procera* and FACA, a mixture of these two plants

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ABSTRACT

Root bark extracts from Z*anthoxylum zanthoxyloides* Lam (Rutaceae) and *Calotropis procera* Aït (Asclepiadaceae) were used in Burkina Faso folk medicine for several diseases particularly sickle cell anemia. Authors reported here vasorelaxant effect of these plants on rat isolated aorta. *Z. zanthoxyloïdes* (cumulative addition) produces a concentration-dependent relaxation on the aorta. Maximal effects are respectively of 60.34 ± 2.34 and 100% in the presence and in the absence of endothelium. *C. procera* extract induces a more relaxing effect on endothelium-denuded aorta (Emax = 59.78 ± 2.13% and 100% in presence and absence of endothelium respectively). FACA, the mixture of these two plants also induces vasorelaxation (Emax = 100%), with better effect in the presence of endothelium (EC_{50} = 2.76 ± 0.38 mg/mL and 4.90 ± 0.69 mg/mL in presence and absence of endothelium respectively). Endothelium-dependent vasodilator effect of FACA was inhibited by L-NAME; this clearly indicates that NO is involved in aorta relaxation process by FACA. Taken together this study revealed that FACA and its components possess vasodilator effect. This vascular property of FACA may be involved in the amendment of sickle cell crisis through inhibition of vaso-occlusion process.

Keywords: Sickle cell anemia, phytotherapy, rat aorta, endothelium.

INTRODUCTION

Sickle cell anemia is a genetic hemoglobinopathy due to a structural anomaly of hemoglobin (Koffi et al., 2002). The therapeutic problem persists (Sangare et al., 1990). Treatment of this disease remains an important process for the African populations because of the heaviness of the care, the high cost of drugs and immobilization of families during hospitalization of patient.

A mixture of root bark extracts from *Zanthoxylum zanthoxyloïdes* Lam (Rutaceae) and *Calotropis procera* aït (Asclepiadaceae) showed clinical effectiveness in the amendment of sickle cell anemia crisis at the

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Hospital of Ouagadougou (Guissou et al., 1995; Dembele, 2001). Antisickling activity of *Z. xanthoxyloïdes* was reported by Osoba et al. (1989). Furthermore studies described three isomeric divanilloylquinic acids isolated from root bark of *Z. zanthoxiloïdes* (Ouattara et al., 2004), with interesting antisickling properties (Ouattara et al., 2009). Extracts from *C. Procera* showed anti-inflammatory and analgesic effects (Ouedraogo et al., 2003; Kumar and Roy, 2009; Tour and Talele, 2011), and proteins from latex of this plant inhibited septic shock due to *Salmonella typhimurium* (Lima-Filho et al., 2010). These revealed properties of the mentioned plants were in favor of crisis amendment.

However the vaso-occlusive crisis of sickle cell anemia painful remains one of the principal symptoms (Girot, 1998; Zittoun et al., 1998; Solary and Belon, 1999). In the vaso-occlusive crisis, sickle cell inhibitors and vasodilators together with blood transfusion are used for the treatment purposes (Bouyer, 1968; Solary and Belon, 1999). So the purpose of the present study was to determine the possible vascular properties of FACA and its implication in the amendment of the sickle cell crisis.

MATERIALS AND METHODS

Plant and extract

Root barks of *C. procera* and *Z. zanthoxyloïdes* were used. Samples (N° 6139 MADSON JE. for *C. procera a*nd N° 0932 for *Z. zanthoxyloïdes*) were deposited in the herbarium of Vegetal Production Department at the National Centre of Scientific Research and Technology in Burkina Faso. Roots of *C. procera* were collected in Linoghin, locality situated at about fifty kilometers in the East of Ouagadougou (Burkina Faso). Z. *zanthoxyloïdes* was collected in Niangoloko, locality situated at 500 km from Ouagadougou, in the West of Burkina Faso. It is a zone of savanna and harvests took place in the dry season. The root barks were removed, cleaned, air dried and then pulverized.

Powder of FACA was obtained by mixture of the two powders at well defined proportions. The preparation, conditioning and the conservation are carried out by IRSS production facility (U-Pharma).

Each extraction consisted by mixing 250 g of powder of drug with 1000 ml of distilled water. The mixture was left to macerate at ambient temperature (30 °C) and under magnetic agitation, and then filtered. The duration of the maceration was of 1 hour 30 minutes for *C. procera* and 12 hours for *Z. zanthoxyloïdes* and FACA. The filtrate obtained was centrifuged at 2000 turns per minutes during five minutes. The supernatant obtained (aqueous extracted) was frozen and then lyophilized at -50 °C. The powders obtained (lyophilizes) were then conditioned in bottles and stored in a desiccators.

Aortic preparation and mounting

Male Wistar rats (*Mou norvegicus albinus*) (12-14 weeks old) were used for the study. Rats were anaesthetized with pentobarbital (60 mg/kg, i.p.) mixed with 500 UI of heparin. The thoracic aorta was removed and mounted in physiological salt solution as previously described (composition in mM: NaCl 119, KCl 4.7, $CaCl_2$ 1.25, $MgSO_4$ 1.17, KH_2PO_4 1.18, $NaHCO_3$ 25 and glucose 11) (Andriambelson et al., 1997), maintained à 37 °C and continuously bubbled with 95% O_2 -5% CO_2 mixture. Aortic rings with and without functional endothelium were pre-contracted to the same tension (i.e. 80% of maximal response obtained in vessel with functional endothelium) with phenylephrine (Phe) 10^{-6} and 5×10^{-8} M respectively. When the contraction reached a steady state,

increased concentrations of extracts were added cumulatively.

In order to characterize the involvement of nitric oxide (NO) and cyclooxygenase (COX) products, some arteries with functional endothelium were exposed to a specific NO synthase (NOS) inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME: 3×10^{-4} M) or to a non specific COX inhibitor indomethacin (10^{-5} M), added to the bath 15 min before Phe. In the case of L-NAME, the concentration of Phe was adjusted in order to obtain the same level of pre-contraction as in the absence of L-NAME.

Drugs and chemicals

NaCl, $NaHCO_3$, KCl, KH_2PO_4, $MgSO_4$, $CaCl_2$, glucose (Sigma Chemical Co, Grenoble, France). Phenylephrine, Indomethacin, L-NAME (Sigma Chemical Co, Grenoble, France). Indomethacin (10^{-5} M) was dissolved in (5 % w/v) $NaHCO_3$.

Statistical analysis

All data were expressed as mean ± SEM. Mean ± SEM, concentration-response curves, and EC_{50} were determined by graphPad Prism version 5.00. Two ways ANOVA and Student's *t*-test were used to determine significant differences between groups. Mean values were considered significantly different when $P<0.05$.

RESULTS

Phe 10^{-6} M induced a constant contraction (2.12 ± 0.15 g; n= 8) on rat aorta. Aqueous extract from *Z. zanthoxyloïdes* (cumulative addition) produced a concentration-dependent relaxation on the aorta with endothelium. Endothelium removal enhances *Z. zanthoxyloïdes* vasodilator effect (Figure 1 A). For this extract the values of maximal effect were respectively of 60.34 ± 2.34 and 100% either in the presence or in the

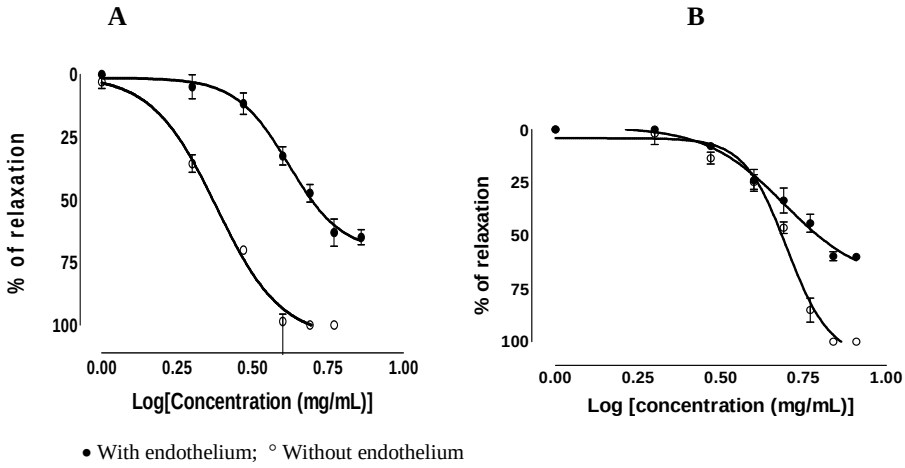

Figure 1: Cumulative concentration-response curves to aqueous rootbark extract from *Z. zanthoxyloides* (A) and *C. procera* (B) on isolated rat aorta previously contracted with phenylephrine (10^{-6} M) in the presence or in the absence of endothelium (A) or with L-NAME and indomethacin treatment (B).
Each point represents the mean ± S.E.M. of 5 to 6 rats.

absence of endothelium respectively. Figure 1B shows similar results with the aqueous extract from *C. procera*. This extract induced a more relaxing effect on endothelium-denuded aorta. Effects from these two extracts are thus partly dependent of the presence of endothelium. In this assay the low concentrations of C. *procera* enhances the vasoconstriction effect of phenylephrine of 21.62 ± 2.97% thus in the presence of endothelium.

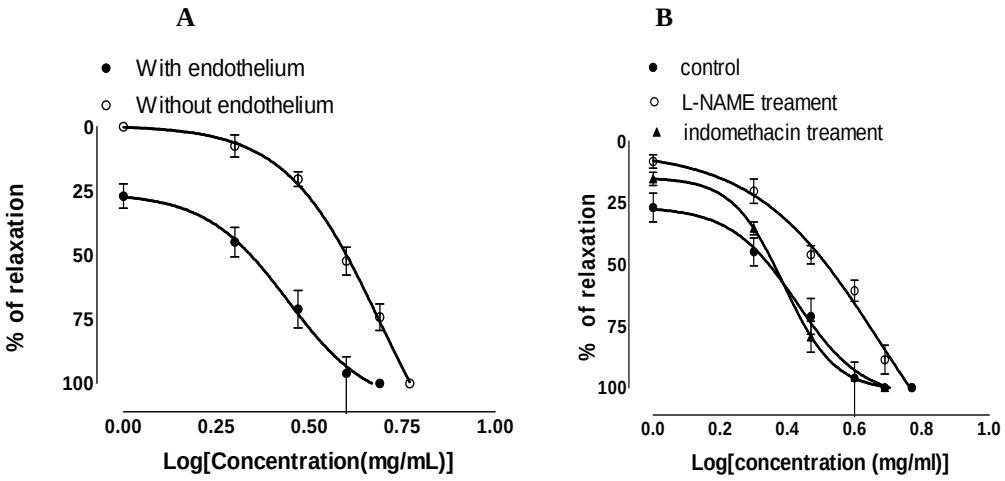

Figure 2: Cumulative concentration-response curves to aqueous extract from FACA on isolated rat aorta, previously contracted with phenylephrine (10^{-6} M) in the presence or in the absence of endothelium (A) or with L-NAME and indomethacin treatment (B).

Each point represents the mean ± S.E.M. of 5 to 6 rats.

Table 1: EC_{50} values for each treatment.

Treatment	EC_{50} (mg/mL)
C. procera with endothelium	--
C. procera without endothelium	5.01 ± 0.35
Z. zanthoxyloïdes with endothelium	--
Z. zanthoxyloïdes without endothelium	2.39 ± 0.25
FACA with endothelium	2.76 ± 0.38[a]
FACA without endothelium	4.90 ± 0.69
FACA + L-NAME	4.76 ± 0.21[b]
FACA + indomethacin	2.44 ± 0.17

a: P<0.001 = FACA with endothelium versus FACA without endothelium

b: P<0.001 = FACA with endothelium versus FACA with L-NAME

For the mixture (FACA) cumulative administration induced concentration-dependent relaxation of rat aorta (Figure 2 A); the vasodilator effect partly depends on the endothelium (see IC_{50} in Table 1). We thus examined NO and COX metabolites relative contribution in the relaxing effect by FACA. The pretreatment of the aorta in the presence of endothelium with the L-NAME (3.10^{-4} M) inhibited the vasorelaxant effect of FACA (Figure 2 B). On the other hand the presence of indomethacin (10^{-5} M) did not influence the vasorelaxant effect of FACA. Compared to the two plants extracts when taking alone, FACA had better vasorelaxation effect in the model with endothelium (Table 1).

DISCUSSION

C. procera produced significant vasodilator activities which were reduced in presence of endothelium. *C. procera* spasmolytic activities were already demonstrated on non vascular muscle (Mossa et al., 1991; Ouedraogo et al., 2003; Moustafa et al., 2010). But low concentrations of root bark (Ouedraogo et al., 2003) and aerial part extract of *C. procera* increased duodenum contraction via muscarinic receptor stimulation (Moustafa et al., 2010). These data indicate that *C. procera* activities on muscle are function of extract concentration, the part of plant used and the Kind of muscles.

The second plant used in FACA composition, *Z. zanthoxyloïdes* also reduces phenyephrine vasoconstriction properties. The vasorelaxant activity of the two plants taken alone is better in the model without endothelium. Thus, these plants seem to act preferentially directly on smooth muscle rather than via endothelium. FACA does not seem to be more efficient than *Z. zantholoïdes* and *C. procera* taken alone in the model without endothelium: when comparing the EC_{50} the vasorelaxant effect of *Z. zanthoxyloïdes* seems more effective than the FACA in this model.

However, FACA is a powerfull vasodilator in the presence of endothelium. This vasodilatory effect can be in profit for sickle cell crisis due to vaso-occlusion (Bouyer, 1968; Solary and Belon, 1999). In the presence of endothelium, FACA also showed better effect than *C. procera* and *Z. zanthoxyloïdes* taken alone, indicating synergic effect of these two plants. Guissou et al. (1995) revealed synergic effect of this mixture for the management of sickle cell disease at Hospital of Ouagadougou (Burkina Faso). Our results thus confirm the interest of the mixture. This study also revealed an endothelium-dependent vasodilator effect of FACA. Some mechanisms of regulation of vascular tone depend on Nitric Oxide (NO) and prostacyclin (Moncada and Higgs, 1988) from endothelium. Inhibition by the L-NAME clearly indicates that NO is involved in the aorta relaxation process induced by FACA.

Conclusion

The present study revealed vasorelaxant activity of FACA, a sickle cell anemia phytomedicine. This effect may be involved in FACA mechanisms of action for the management of this pathology. These arguments confirm the ethnomedicine use of this recipe.

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